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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: G01N	A2	(11) International Publication Number: WO 97/18454 (43) International Publication Date: 22 May 1997 (22.05.97)
(21) International Application Number: PCT/US96/18567 (22) International Filing Date: 15 November 1996 (15.11.96) (30) Priority Data: 60/006,838 16 November 1995 (16.11.95) US 08/594,031 30 January 1996 (30.01.96) US (71)(72) Applicant and Inventor: THOMPSON, Timothy [US/US]: 4835 Jason Street, Houston, TX 77096 (US). (74) Agents: REMENICK, James et al.; Baker & Botts, L.L.P., The Warner, 1299 Pennsylvania Avenue, N.W., Washington, DC 20004 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: METHOD FOR IDENTIFYING METASTATIC SEQUENCES (57) Abstract The invention relates to methods for the identification of metastatic sequences. Cells from a cell line or an animal tissue are treated to form a cell line predisposed to metastasis. Treated cells are implanted in an animal of a primary site and incubated for a period of time sufficient for the cells to proliferate and develop metastases at secondary sites. Expressed sequences from cells at the primary and secondary sites are amplified by differential display polymerase chain reaction and compared. Differentially expressed sequences are identical and can be cloned and sequenced. These sequences can be used as probes in the diagnosis of metastatic disorders, as probes to isolate metastatic sequences and as a therapeutic agent.		

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METHOD FOR IDENTIFYING METASTATIC SEQUENCES

Rights in the Invention

This invention was made in part with United States Government support under grant number CA350129, awarded by the National Cancer Institute, National Institute of Health and the United States Government has certain rights in the invention.

Background

1. Field of the Invention

The present invention relates to methods for the identification and isolation of metastatic sequences, to diagnostic probes and kits which contain metastatic sequences and to therapeutic treatments for neoplastic disorders based on metastatic sequences.

2. Description of the Background

The development of higher organisms is characterized by an exquisite pattern of temporal and spatially regulated cell division. Disruptions in the normal physiology of cell division are almost invariably detrimental. One such type of disruption is cancer, a disease that can arise from a series of genetic events.

Cancer cells are defined by two heritable properties, uncontrolled growth and uncontrolled invasion of normal tissue. A cancerous cell can divide in defiance of the normal growth constraints in a cell leading to a localized growth or tumor. In addition, some cancer cells also gain the ability to migrate away from their initial site and invade other healthy tissues in a patient. It is the combination of these two features that make a cancer cell especially dangerous.

An isolated abnormal cell population that grows uncontrollably will give rise to a tumor or neoplasm. As long as the neoplasm remains in a single location, it is said to be benign, and a complete cure may be expected by removing the mass surgically. A tumor or neoplasm is counted as a cancer if it is malignant, that is, if its cells have the ability to invade surrounding tissue. True malignancy begins when the cells cross the basal

lamina and begin to invade the underlying connective tissue. Malignancy occurs when the cells gain the ability to detach from the main tumor mass, enter the bloodstream or lymphatic vessels, and form secondary tumors or metastases at other sites in the body. The more widely a tumor metastasis, the harder it is to eradicate and treat.

As determined from the epidemiological and clinical studies, most cancers develop in slow stages from mildly benign into malignant neoplasms. Malignant cancer usually begins as a benign localized cell population with abnormal growth characteristic called a dysplasia. The abnormal cells acquire abnormal growth characteristics resulting in a neoplasia characterized as a cell population of localized growth and swelling. If untreated, the neoplasia *in situ* may progress into a malignant neoplasia. Several years, or tens of years may elapse from the first sign of dysplasia to the onset of full blown malignant cancer. This characteristic process is observed in a number of cancers. Prostate cancer provides one of the more clear examples of the progression of normal tissue to benign neoplasm to malignant neoplasm.

The walnut-sized prostate is an encapsulated organ of the mammalian male urogenital system. Located at the base of the bladder, the prostate is partitioned into zones referred to as the central, peripheral and transitional zones, all of which surround the urethra. Histologically, the prostate is a highly microvascularized gland comprising fairly large glandular spaces lined with epithelium which, along with the seminal vesicles, supply the majority of fluid to the male ejaculate. As an endocrine-dependent organ, the prostate responds to both the major male hormone, testosterone, and the major female hormones, estrogen and progesterone. Testicular androgen is considered important for prostate growth and development because, in both humans and other animals, castration leads to prostate atrophy and, in most cases, an absence of any incidence of prostatic carcinoma.

The major neoplastic disorders of the prostate are benign enlargement of the prostate, also called benign prostatic hyperplasia (BPH), and prostatic carcinoma; a type of neoplasia. BPH is very common in men over the age of 50. It is characterized by the presence of a number of large
5 distinct nodules in the periurethral area of the prostate. Although benign and not malignant, these nodules can produce obstruction of the urethra causing nocturia, hesitancy to void, and difficulty in starting and stopping a urine stream upon voiding the bladder. Left untreated, a percentage of these prostate hyperplasia and neoplasias may develop into malignant prostate
10 carcinoma.

In its more aggressive form, transformed prostatic tissues escape from the prostate capsule and metastasize invading locally and throughout the bloodstream and lymphatic system. Metastasis, defined as tumor implants which are discontinuous with the primary tumor, can occur
15 through direct seeding, lymphatic spread and hematogenous spread. All three routes have been found to occur with prostatic carcinoma. Local invasions typically involve the seminal vesicles, the base of the urinary bladder, and the urethra. Direct seeding occurs when a malignant neoplasm penetrates a natural open field such as the peritoneal, pleural or pericardial
20 cavities. Cells seed along the surfaces of various organs and tissues within the cavity or can simply fill the cavity spaces. Hematogenous spread is typical of sarcomas and carcinomas. Hematogenous spread of prostatic carcinoma occurs primarily to the bones, but can include massive visceral invasion as well. It has been estimated that about 60% of newly diagnosed
25 prostate cancer patients will have metastases at the time of initial diagnosis.

Surgery or radiotherapy is the treatment of choice for early prostatic neoplasia. Surgery involves complete removal of the entire prostate (radical prostatectomy), and often removal of the surrounding lymph nodes, lymphadenectomy. Radiotherapy, occasionally used as adjuvant therapy,
30 may be either external or interstitial using ¹²⁵I. Endocrine therapy is the

treatment of choice for more advanced forms. The aim of this therapy is to deprive the prostate cells, and presumably the transformed prostate cells as well, of testosterone. This is accomplished by orchiectomy (castration) or administration of estrogens or synthetic hormones which are agonists of luteinizing hormone-releasing hormone. These cellular messengers directly inhibit testicular and organ synthesis and suppress luteinizing hormone secretion which in turn leads to reduced testosterone secretion by the testes. Despite the advances made in achieving a pharmacologic orchiectomy, the survival rates for those with late stage carcinomas are rather bleak.

10 Summary of the Invention

The present invention overcomes the problems and disadvantages associated with current strategies and designs and provides new methods for the identification of sequences related to metastasis.

One embodiment of the invention is directed to methods for the identification of a metastatic sequence. One or more oncogenic sequences are transfected into a cell to form a transfected cell. The transfected cell is introduced into a primary site of a host animal to establish a colony which is incubated in the animal for a period of time sufficient to develop both a primary tumor and a malignant tumor. Expressed sequences are harvested from the primary tumor and the metastasis. Harvested sequences are compared to each other and to non-metastatic cells to identify sequences related to metastasis. Dominant metastatic genes are genes whose expression leads to metastasis. Such genes are typically expressed at high levels in metastatic cells and not significantly expressed in normal or nonmetastatic cells. Recessive metastatic genes, genes whose expression prevents metastasis, may be selectively expressed in normal and nonmetastatic cells and absent in metastatic cells. Dominant and recessive metastatic genes may act directly or act pleiotropically by enhancing or

inhibiting the expression or function of other dominant and recessive metastatic genes.

Another embodiment of the invention is directed to methods for identifying metastatic sequences. A mammalia cell is treated with a
5 metastatic agent and the treated cell is implanted into a primary site of a host mammal. The host animal is maintained for a period of time sufficient for the cells to proliferate and to develop a metastasis at a secondary site. Expressed sequences from cells of the primary site and cells of the secondary site are reverse transcribed into cDNA by differential display polymerase
10 chain reaction to identify differentially expressed sequences.

Another embodiment of the invention is directed to sequences isolated by the methods of the invention. Sequences may be in the form of DNA, RNA or PNA. The nucleic acid may be single-stranded or double-stranded. Single stranded nucleic acid may be in the form of a sense strand
15 or an antisense strand. In addition, the sequence may be part of a homologous recombination vector designed to recombine with another metastatic sequence.

Another embodiment of the invention is directed to a method for treating a neoplastic disorder comprising administering a
20 pharmaceutically effective amount of a metastatic nucleic acid to a patient. The nucleic acid may be single-stranded in the sense or the antisense direction. Alternatively, the nucleic acid may be packaged in a viral vector such as, for example, a retroviral, a vaccinia or an adenoviral vector. Administration may be performed by injection, pulmonary absorption,
25 topical application or delayed release of the nucleic acid along with a pharmaceutically acceptable carrier such as water, alcohols, salts, oils, fatty acids, saccharides, polysaccharides and combinations thereof.

Another embodiment of the invention is directed to a kit for detecting of the presence or absence of a metastatic sequence.

Other objects and advantages of the invention are set forth in part in the description which follows, and in part, will be obvious from this description, or may be learned from the practice of the invention.

Description of the Drawings

- 5 Figure 1 Schematic showing two paths in the multistep progression to cancer.
- Figure 2 Staining of primary tumor (A) and metastatic deposit (B) from the lung of the same animal
- Figure 3 Staining of normal human prostate (A), moderately
10 differentiated human prostate tumor (B and C), and poorly differentiated prostate tumor (D).
- Figure 4 Schematic of method for isolating a metastatic gene from a gene ablated mouse strain.
- Figure 5 Schematic showing method to establish a tumor and a
15 metastatic transplant from fetal tissue(A) and from cell lines and tumors (b).
- Figure 6 Isolation and characterization of nmb gene expression by DD-PCR and RNA blot in primary and metastatic cells.
- Figure 7 Differential expression of multiple genes is determined by
20 DD-PCR and RNA blot of primary and metastatic cells.
- Figure 8 Caveolin identified as a differentially expressed gene by DD-PCR.
- Figure 9 Differential expression of genes isolated by DD-PCR confirmed by RNA blots.
- 25 Figure 10 RNA blot analysis of total tumor mRNA using clone 29 GADPH probes.
- Figure 11 RNA blot of three independent MPR metastatic tumors and 5 MPR non-metastatic tumors.
- Figure 12 Nucleotide sequences of metastatic nucleic acids.

Figure 13 Characterization of metastatic sequences isolated.

Figure 14 Immunohistological staining of primary and metastatic human prostate tumors using anti-caveolin antibodies.

Description of the Invention

5 As embodied and broadly described herein, the present invention is directed to methods for identifying metastatic sequences, to the metastatic sequences identified, to methods for the detection, diagnosis and treatment of disorders related to metastasis, and to diagnostic kits which comprise these sequences.

10 The ability of cancers to metastasize makes tumors difficult to eradicate by any means. Malignant cancer involves a multistage progression from, for example, normal tissue through hyperplasia, early adenoma, early carcinoma and finally to a metastatic tumor (Figure 1). Cells of a typical tumor loosen their adhesion to their original cellular neighbors and cross the
15 basal lamina and endothelial lining to enter the body's circulation. Once in circulation, the metastatic cell exits from the circulation to disseminate throughout body and proliferate in a new environment.

Like the initial oncogenic event, the ability of a cell to metastasize requires additional mutational or epigenetic changes. An
20 understanding of the molecular mechanisms of metastasis allow for the design of treatments to inhibit metastasis. Knowledge of stage specific gene expression for neoplastic disorders allows for early detection and typing of tumors. With early detection and typing, proper treatment may be administered to a patient with the neoplastic disorder earlier, which will lead
25 to a higher probability of a complete cure.

For human prostate tumors, the study of stage specific tumors is difficult, if not impossible, as cell lines are extremely difficult to grow and it is rare that tissue becomes available from the primary tumor as well as metastatic disease from the same patient. This problem is exacerbated

because of the infrequent biopsy of metastatic deposits in concordance of isolation of material from the primary tumor. Furthermore, the growth of cell lines from malignant prostates has proved to be problematic over the last few decades. This is evidenced by the lack of cell lines from prostate cancer
5 obtained under any conditions.

One embodiment of the invention is directed to a method for identifying a metastatic sequence. A mammalian cell is transformed into a pre-neoplastic or neoplastic state or phenotype by transfection with one or more oncogenic sequences. Alternatively, or in addition to transfection, the
10 mammalian cell may be treated with an agent or subjected to a condition that potentiates the metastatic character of the cell or predisposes the cell to metastasis. The transfected or treated cell is implanted into a host animal at a primary site and grown for a period of time sufficient to develop a metastasis at a secondary site. Expressed sequences from cells of the
15 primary site and cells at the secondary site are amplified by differential display polymerase chain reactions. PCR products from these reactions are compared and the metastatic sequence identified by alteration in the levels or patterns of the resulting products.

Mammalian cells from a wide variety of tissue types and
20 species are suitable for transfection or treatment including surgically obtained or primary or immortalized cells and cell lines. Cells may be from humans or primates, mice, rats, sheep, cows, rabbits, horses, pigs or guinea pigs or from transgenic or xenogeneic host mammals. Cells may be obtained from adult, juvenile or fetal tissue, and used directly from the mammal, from
25 cryogenically preserved samples, or after culturing *in vitro* or *in vivo* for a period of time. *In vitro* culturing typically involves tissue culture conditions (e.g. 37°C; 5% CO₂) while *in vivo* culturing may involve successive passage of cells through host animals such as, for example, mice or rabbits. Cells passed *in vivo* may be obtained from sites proximal or distal to the site of
30 implantation. The tissue type from which the cells are derived or obtained

may be any tissue which is susceptible to transfection or other treatment including, for example, urogenital tissues, epithelial cells, hepatic cells, fibroblasts lymphatic tissues, hematopoietic cells, cells of the immune system, cells of the gastrointestinal system and cells of the nervous system.

5 Cell types useful for the identification of metastatic sequences related to prostate cancer include cells and cell lines of the fetal prostate lineage from normal or transgenic animals, and cells from normal or reconstituted prostate tissue. One method of generating reconstituted prostate cells is to isolate fetal prostate tissue and microdissect the fetal
10 prostate epithelium away from fetal mesenchyme. Fetal prostate epitheliums may be genetically manipulated before reassociation with fetal mesenchyme (Figure 5A). Genetic manipulation involves treatment or transfection with a metastatic agent or a nucleic acid sequence that affects neoplastic or metastatic potential of the cell. Reassociation of fetal epithelium and
15 mesenchyme is performed by implanting epithelium tissue within a pocket of mesenchyme tissue. After manipulation, cells are reimplanted into a mammalian host in a similar manner as other cells, such as reimplantation into or under the renal capsule.

Mammalian cells may be transfected by a variety of
20 techniques, all of which are well-known to those of ordinary skill. Direct methods involve the introduction of genetic material into the nucleus of a cell by injection. These techniques include high velocity projectile injection, microinjection, and electroporation. Indirect methods, involving the active or passive uptake of the genetic information by the cell. Indirect techniques
25 include transduction with recombinant vectors, and chemical or physical treatments such as calcium phosphate uptake, lipofection or dextran sulfate transfection. Chemical techniques rely on chemical carriers to introduce nucleic acids into a cell. These methods, for example, utilize unilamellar phospholipid vesicles (*e.g.* liposomes) loaded with DNA (or RNA). The
30 approach relies on the fusion of the DNA containing vesicles with the

plasma membrane of the recipient cells. After entry, DNA traverse the cytoplasm and enter the nucleus. Another lipofection technique uses a synthetic cationic lipid such as N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA). DOTMA spontaneously associates
5 with nucleic acids and forms unilamellar vesicles upon sonication. Genetic material is incorporated into these vesicles and subsequently transfected into the cell. Calcium phosphate co-precipitation involves mixing of purified nucleic acid with buffers containing phosphate and calcium chloride which results in the formation of a fine precipitate. Presentation of this precipitate
10 to cells results in incorporation of the nucleic acid into cellular genome. Other chemicals, such as DEAE dextran or polybrene, when present in media with nucleic acids, can also cause the transfection of mammalian cells.

Physical methods of transfection rely on electric fields, needles
15 and particles to enable nucleic acids to traverse the cellular membrane. Electric field mediated DNA transfection, commonly called electroporation, is based on the principle that membranes, when subjected to an electric field, undergo a reversible breakdown resulting in pores large enough to permit the passage of nucleic acids. In micro-projectile mediated gene transfer, micro-
20 projectiles of subcellular dimensions are coated with nucleic acid and propelled at high velocity into a cell using a particle gun. The nucleic acid is introduced into the nucleus directly when the particles impinge upon the nucleus. In microinjection, nucleic acid is injected directly into the nucleus of a cell with a needle. Lasers have also been used to introduce minute holes
25 in cellular membrane to allow introduction of nucleic acids. All these methods may be used for transfection and the selection of the method will depend on the cell type, the desired transfection efficiency and the equipment available.

The efficiency of transfection may be monitored and enhanced
30 by the co-transfection of a selectable marker. If a marker is co-transfected

with a genetic construct, positively transformed cells may be separated from nontransformed cells by chemical selection. The efficiency of transfection will be increased in most cases because the chemicals will selectively kill non-transfected cells. The number of transfected cells may also be
5 monitored by analyzing the degree of chemical resistance of the transfected cells. Markers commonly used for selection purposes include, for example, nucleic acids encoding dihydrofolate reductase, metallothionein, CAD, adenosine deaminase, adenylate deaminase, UMP synthetase, IMP 5'-dehydrogenase, xanthine-guanine phosphoribosyltransferase, mutant
10 thymidine kinase, mutant HGPRTase, thymidylate synthetase, P-glycoprotein 170, ribonucleotide reductase, glutamine synthetase, asparagine synthetase, arginosuccinate synthetase, ornithine decarboxylase, HMG-CoA reductase, N-acetylglucosaminyl transferase, theronyl-tRNA synthetase, sodium or potassium dependent ATPase or derivatives or mutants of these
15 nucleic acids. Markers may be used individually or in combination. Chemicals useful for selection include methotrexate, cadmium, PALA, Xyl-A, adenosine, 2'-deoxycoformycin, adenine, azaserine, coformycin, 6-azauridine, pyrazofuran, mycophenolic acid, limiting xanthine, hypoxanthine, aminopterin, thymidine, 5-fluorodeoxyuridine, adriamycin,
20 vincristine, colchicine, actinomycin D, puromycin, cytocholasin B, emetine, maytansine, Bakers' antifolate, aphidicolin, methionine sulfoximine, β -aspartyl hydroxamate, albizziin, canavanine, α -difluoromethylornithine, compactin, tunicamycin, borrelidin, ouabain, and derivatives and analogs and combinations of these chemicals. Some chemicals, such as
25 methotrexate, may be used individually while other chemicals, such as HAT (hypoxanthine, aminopterin and thymidine), need to be used in combination to be effective.

The oncogene transfection efficiency, the fraction of live cells tranfected by an oncogene, may be indirectly enhanced by chemical
30 selection for a co-transfected marker. An oncogene is a sequence which can

predispose, or induce the cell into a pre-neoplastic or neoplastic condition or otherwise enhance the metastatic potential of the cell. Sequences with these properties are referred to as oncogenes and include *abl*, *ah1*, *akt*, *bcl*, *crk*, *dsi*, *erb*, *ets*, *evi*, *fes/fps*, *fim*, *fis*, *fgr*, *flv*, *fms*, *fos*, *gin*, *gli*, *int*, *jun*, *kit*,
5 *mas*, *lck*, *met*, *mil/raf*, *mis*, *mlv*, *mos*, *myb*, *myc*, *neu*, *onc*, *pim*, *raf*, *ras*, *rel*,
ros, *seq*, *sis*, *ski*, *spi*, *src*, *tcl*, *thy*, *trk*, and *yes*. Some oncogenes, such as *ras*, are oncogenic when mutated. Other oncogenes, such as *myc*, are oncogenic when overexpressed or underexpressed. Many oncogenes represent members of multigene families or homologs families. Homologs are proteins that
10 have similar primary, secondary or tertiary structures. Genes may differ in nucleic acid sequence or encoded peptide sequence and still be homologs when the encoded polypeptides have similar spatial folding. Many oncogenes can be classified into dominant oncogenes and recessive oncogenes. One or more dominant oncogenes can confer a neoplastic or pre-
15 neoplastic phenotype to a cell. One or more recessive oncogenes, when silenced, may also confer a neoplastic or preneoplastic phenotype. Gene silencing is performed by transfecting cells with nucleic acids which cause genetic ablation or by antisense suppression.

While any oncogene may be used, the preferred oncogenes are
20 those that are normally associated with metastasis such as a metastasis specific gene. Such genes include for example, *TGF- β 1*, *Cyclin D1* *p21*, *p34*, *p53*, *lysyl oxidase*, *caveolin*, *actin binding protein*, *ubiquitin activating enzyme E1*, *nmb* or *α -actinin 3*. Metastatic-specific genes may be used individually or in combination with other oncogenes.

25 The metastatic potential of a cell may be altered, for example, by gene ablation with a sequence specific for a recessive oncogene. Recessive oncogene are those genes which encode products which can suppress oncogenesis and metastasis. A gene ablation sequence can be designed to specifically suppress a recessive oncogene. Ablation may
30 include pre-transcriptional inhibition such as homologous recombination

with endogenous recessive oncogenes and post transcriptional inhibition such as the expression of antisense oncogenes to suppress translation. Gene ablation sequences may be targeted towards well known recessive oncogenes such as, for example, the retinoblastoma gene (Rb) or Bcg. Other candidates
5 for ablation include metastatic genes previously isolated by the invention such as, for example, TGF- β 1, cyclin D1, p21, p34, p53, lysyl oxidase, caveolin, actin binding protein, ubiquitin activating enzyme E1, nmb or α -actinin-3. The effects of ablating a recessive oncogene may include oncogenesis and metastases.

10 Alternatively, or in addition to transfecting the mammalian cell may be treated with an agent, either before or after transfection, that alters the expression of the cell's nucleic acids. Treatment may comprise contacting the cells with one or more agents which affect the neoplastic (*e.g.* neoplastic agents; phorbol esters), metabolization (*e.g.* metabolic agents),
15 metastatic (*e.g.* metastatic agents), differentiation (*e.g.* differentiation agents; retinoic acid), activation or proliferation (*e.g.* growth factors) of the cell. Agents which can alter gene expression include chemicals such as benzanthracycline (BA), dimethyl benzanthracycline (DMBA) or 5-azacytidine. Alternatively, treatment may also comprise altered conditions such as
20 hypoxia which involves subjecting a cell to a reduced oxygen content, exposable to radiation or other stresses to the cell.

Treatment may be *in vitro* or *in vivo* and may include for example, direct or indirect induction or suppression of well known oncogenic sequences and genes isolated by the invention such as, for example, TGF- β 1,
25 Cyclin D1, p53, lysyl oxidase, caveolin, actin binding protein, ubiquitin activating enzyme E1, nmb, α actinin 3, and p34. Gene expression induction includes transfecting expression vectors encompassing coding regions of the gene. Gene repression comprises introducing a gene ablation sequence or a repressor of the gene to the cell.

Cells which have one or more genes ablated may also be used. For example, a metastatic suppressor gene may be ablated to prevent inhibition to metastases. A useful gene for ablation is a gene capable of affecting the phenotype and behavior of a cell or tumor. For example, with
5 prostate tumors, suitable genes include both well known genes and genes isolated by the methods of the invention such as for example, *TGF- β 1*, *Cyclin D1*, *p21*, *p34*, *p53*, *lysyl oxidase*, *caveolin*, *actin binding protein*, *ubiquitin activating enzyme E1*, *nmb* and *α actinin 3*. Genetic ablation (gene knockout) refers to a process of silencing the expression of a particular gene
10 in a cell. The silencing process may include, for example, gene targeting or antisense blocking. Gene targeting refers to a process of introducing a nucleic acid construct into a cell to specifically recombine with a target gene. The nucleic acid construct inactivates the targeted gene. Inactivation may be by introduction of termination codons into a coding region or introduction
15 of a repression site into a regulatory sequence. Antisense blocking refers to the incorporation into a cell of expression sequences which directs the synthesis of antisense RNA to block expression of a target gene. Antisense RNA hybridizes to the mRNA of the target gene to inhibit expression.

The host animal is preferably the same species as the
20 implanted cell. In cases of xenogeneic transplants, the host may be immunocompromised genetically or by treatment with drugs such as immunosuppressants. A host may be immunocompromised genetically by breeding such as with nude mice or severe combined immunodeficient (SCID) mice. A host may also be immunocompromised by chemical or
25 irradiation methods. An additional route to immunocompromise a host is to use transgenic technology to introduce an immunosuppressing gene or to introduce a foreign antigen gene. An immunosuppressing gene is a gene that affects the efficiency of the immune system such as a gene which inhibits the formation of cells of the B cell or T cell lineage. A foreign antigen gene,

when expressed, may cause the host to tolerate the antigens in a xenogeneic transplant and not mount an immune response.

Cells may be implanted into any primary site in a host animal, such as, for example, subcutaneous implantation, intravenous injection, or
5 implantation into the abdominal cardiac, chest, pulmonary, thoracic or peritoneal cavity. Using techniques known to those of ordinary skill in the art, cells can be placed on or in nearly any organ or tissue. Reasons for choosing a site include ease of implant, proximity of similar tissue type, immunoprivileged position and ease of inspection. Metastases migrate
10 from the primary site to one or more secondary sites such as, for example, the lung, kidney, liver, lymph nodes, brain, testis, bone, spleen, ovaries or mammary. Preferred sites include the renal capsule, the testes, the prostate and the ovaries.

To avoid histocompatibility problems, the implant may be
15 placed into a histocompatible host animal. Such problems are generally avoided if the host animal are syngeneic. Alternatively, a non-histocompatible host may be used if the host can be made immunotolerant. Hosts may also be transgenic or immunocompromised animals or genetically matched to the mammalian cells to be introduced. Immunocompromised
20 animals may be derived from established mouse lines such as nude mice or severe combined immune deficiency (SCID) mice, or by treatments such as radiation, chemical, pharmaceutical or genetic targeting. Sufficiently immunosuppressed animals can be made tolerant to xenogeneic transplants.

After implantation the host animal is maintained under normal
25 conditions to develop metastases. Alternatively, the host animal may be subjected to an altered treatment or environmental condition to stimulate or repress metastasis or induce other cellular functions. In metastasis, a sub-population of cells of the implantation site invade and establish one or more secondary colonies in the host animal. The behavior of the implanted cell
30 will depend on the cell type, the transfected sequence and the implantation

location. Typical secondary sites for metastatic colonies include lung, kidney, liver, lymph nodes, brain, testis, spleen, bone, ovary, skin and mammary tissue. Metastatic development times vary from days to weeks even months. Cells with a high metastatic potential tend to progress to metastasis quickly while cells with a low metastatic potential may require very long periods of time that span significant portions of the lifespan of the animal.

The host animal may be analyzed for metastatic development weekly, from one week to 20 weeks to six months, nine months or one year after implantation. For animals with longer lifespans such as sheep, the animal may be inspected yearly from one year on up to ten years for metastatic tumors. Metastases can be detected by examinations such as palpitation, biopsy, imaging, exploratory surgery, CAT scans, autopsy, X-ray and direct observation. In addition, tissue samples may be taken surgically from the host mammal and subjected to histological or other examination for the detection of metastases.

Expressed sequences include mRNA, rRNA, hnRNA, DNA, cDNA and any nucleic acid sequence that is expressed in the cell. These sequences may be amplified by *in situ* techniques or by purification of nucleic acid from collected cells. Expressed sequences may be obtained by extracting nucleic acids from cells before implantation, at the primary site or at the secondary site. Cells collected at these sites may optionally be cultured for a time before nucleic acid extraction. The effects of treatment with gene expression modifying agents or environmental conditions can be ascertained by collecting cells before and after treatment. Treatment may be applied to the cells while the cells are in the host mammal or after the cells are excised and in culture. Nucleic acid are collected from cells using techniques that are well known to those of ordinary skill in the art.

Expressed sequences may be used directly for polymerase chain reaction (PCR) analysis using, for example, the technique of reverse

transcriptase polymerase chain reaction (RT-PCR). Alternatively, RNA may be enriched for mRNA using a poly-A RNA enrichment method. Numerous poly-A RNA enrichment methods exist and are commercially available. Techniques used for poly-A RNA enrichment include oligo-dT columns, oligo-dT magnetic beads, and oligo-dT cellulose. RNA may be further processed into cDNA before analysis by reverse transcription using reverse transcriptase. The cells or the extracted nucleic acid may be preserved, such as by freezing, and analyzed at a later time.

Differential display polymerase chain reactions (DD-PCR) are performed on the expressed sequences using two variable primers which may contain the same or entirely different sequences or an anchor primer and a variable primer. If an anchor primer is used, one anchor primer and one variable primer create a single or a single set of reaction products for each reaction. A complete profile may include 25 or more different PCR reactions per sample wherein each PCR reaction is performed with the same anchor primer and a different variable primer. DD-PCR may also be performed using anchor and variable primers which contain the same sequence. Whether a particular reaction is used depends on whether a difference exists between the products of two PCR reactions using the same primers. When a significant difference exists between the expression sequences amplified, one pair of PCR reactions may be sufficient and informative.

Anchor primers are preferably oligonucleotides with a poly-T sequence at the 5' -terminas and a dinucleotide selected from the group consisting of AA, AG, AC, AT, GA, GG, GC, GT, CA, CG, CC and CT at the 3'-terminas. For example, the sequence may be 5'-TTTTTTTAA-3' or 5'-TTTTTTTAG-3'. The length of the poly-T sequence is typically between about 5 to about 30 bases in length and preferably between about 10 to about 20 nucleotides long. The total length of the anchor primer can vary greatly for each experiment but is preferably between about 7 to about 32 and more

preferably between about 12 and about 22. Differential diagnostic polymerase chain reaction may also be performed using an anchor primer of any sequence and a length between about 5 to about 30, preferably between about 5 to about 20 and more preferably between about 7 to about 12 bases.

5 The variable primer may comprise a random sequence, or a specific sequence such as, for example, a sequence of SEQ ID NO. 1 to SEQ ID NO. 24. Variable primers preferably are oligonucleotides with a length between about 5 to about 30, preferably between about 5 to about 20, and more preferably between about 7 to about 12 bases in length.

10 To enhance detection of the PCR product, the anchor primer or the variable primer, or both, may comprise a detectable moiety. Examples of detectable moieties include radioactive moieties, phosphorescent moieties, magnetic moieties, luminescent moieties, conjugatable moieties or other detectable moiety. A plurality of detectable moieties may be used to
15 enhance detection or to simplify data analysis. Other detectable moieties include conjugatable moieties and molecules which can bind specifically to other molecules which are themselves detectable. Examples of conjugatable moieties include avidin, streptavidin, biotin, antibody, antigen, cell adhesion molecules and other molecules with similar activities. Detectable moieties
20 are preferably labeled nucleotides. A nucleotide may be any natural or synthetic nucleotide or nucleotide analog capable of incorporation into an elongation reaction in a polymerase chain reaction. Labeled nucleotides include nucleotide triphosphates labeled with one or more radioactive atoms such as ^{32}P , ^{33}P , ^3H , ^{14}C and ^{35}S .

25 Products of DD-PCR reactions are compared to detect the metastatic sequence. Comparisons can be performed between expressed sequences from cells at secondary sites with cells at any stage in the method including untreated mammalian cells, transfected or treated mammalian cells, implanted cells or cells obtained from the primary site in the host

animal. DD-PCR products may be analyzed by any method which reliably compares the products of two polymerase chain reactions. Typical analytical methods used for this purpose include polyacrylamide gel electrophoresis, capillary electrophoresis and high pressure liquid chromatography (HPLC).

- 5 Product produced from DD-PCR may be analyzed in double-stranded or single-stranded forms. When the products of the DD-PCR reaction are labeled the sizes and distribution of the products may be monitored and analyzed by following the labels using a radiation monitor or by autoradiography. For example, DD-PCR performed in the presence of
10 radioactive primers or nucleotide triphosphates, can be analyzed by gel electrophoresis, by capillary electrophoresis, or by HPLC. Products are easily monitored by the presence of radioactivity.

- Another method for analyzing and isolating metastatic sequences is to sequence the amplified nucleic acid sequences. Sequencing
15 may be performed using standard methods well known to those of ordinary skill in the art. The resulting sequence may be compared to a sequence database created or well-known, such as Genbank, for identification or for locating homologs. The sequencing information may be used to calculate the physical characteristics of the nucleic acids such as melting temperature
20 and secondary structure. The primary sequence and the physical characteristic may be used to synthesize optimal nucleic acid probes for the detection or staging of metastasis or conditions that are predictive of the presence or absence of the metastatic condition.

- Another embodiment of the invention is directed to a methods
25 for identifying a metastatic sequence. A mammalian cell is pretreated with a metastatic agent to form a population of cells predisposed to metastasize. The treated cells are introduced into a host mammal at a primary site. The host animal is maintained for a period of time sufficient to develop a metastasis at a secondary site. Expressed sequences of cells at the primary
30 site and cells at the secondary site are treated with a genotoxic agent or

subjected to genotoxic conditions. Expressed sequences of the treated cells are amplified by differential display polymerase chain reaction and compared with untreated cells from any previous step to identify the metastasis sequence.

5 The metastatic agent may be a chemical compound, a nucleic acid or a protein that alters the metastatic potential of a cell or relates to or is associated with the metastatic process. Chemical compounds include retinoids such as 4-hydroxyphenyl (4HP). Other agents include the proteins TGF- β 1, Cyclin D1, p21, p34, p53, lysyl oxidase, caveolin, actin binding
10 protein, ubiquitin activating enzyme E1, nmb or α -actinin 3, or their respective genes. The metastatic agent may be a metastatic stimulant or a metastatic suppressant. Metastatic stimulants may be used to enhance the sensitivity of the metastasis sequence detection method. Conversely metastatic suppressants may be used to decrease the sensitivity of the
15 method enabling the selective identification of potent metastasis sequences or sequences specific to a particular tissue type or metastatic disorder. Treatment may comprise direct contact with the metastatic agent or incubation for a period of time. Metastatic agents enhance the metastatic potential of the implanted cells and increase the sensitivity and the speed of
20 the overall method.

 The cells at the primary site and the metastatic cells at the secondary site may be treated with a genotoxic agent *in vivo* or *in vitro*. *In vivo* treatment may comprise injecting genotoxic agents directly into the host mammal or specifically applying the agent with, for example, topical
25 formulations. The cells at the primary site and the secondary site may also be isolated from the host animal and treated with the genotoxic agent in culture. Genotoxic agents are chemical compounds, nucleic acids or proteins that alter gene expression by effecting the nucleic acid genome directly by, for example, chemical modification, or indirectly by, for
30 example, altering components associated with gene expression. Such agents

include, for example, benzanthrane (BA), dimethyl benzanthrane (DMBA) and 5-azacytidine, and may include metastatic agents as well. In addition to or in place of genotoxic agents, the cells may be treated to hypoxic conditions or radiation to alter gene expression. Metastatic sequences identified in these methods may be specific for particular genotoxic agents or conditions.

Another embodiment of the invention is directed to the use of a host animal with an altered genotypic or phenotypic predisposition for metastases. A host animal may be screened for endogenous expression of metastases gene. Examples of metastatic sequences which may be screened for include sequences isolated by the method of the invention, such as, for example, the sequences listed in Figure 12 and Figure 13. Particularly useful metastatic sequences include *TGF- β* . A host animal with reduced levels of a metastatic gene product may be used to isolate novel metastatic genes. Host animals may be screened for reduced levels of metastatic gene expression. In addition, transgenic technology may be use to ablate a metastatic gene in the germline of a host animal.

Another embodiment of the invention is directed to analysis of a cell line before their use as a starting material to isolate metastatic genes in a particular pathway. Analysis is useful in identifying cells, and consequently sequences specific to these cells, which are particularly susceptible or resistant to metastatic transformation. For example, a cell highly predisposed to metastasis may be especially sensitive for detecting metastatic genes. Conversely, a cell showing high resistance to metastasis can be used to isolate especially potent metastatic sequences. One method to analyze susceptibility to metastasis is to determine the cellular response to growth factors or growth inhibitors. Briefly, a control population and a test population of cells are exposed to a growth factor or a growth inhibitor and the cellular response (e.g. proliferation, metabolism) recorded. Cells showing abnormal responses to the growth factor or growth inhibitor may be

used as the starting material for metastatic gene isolation. Cellular response include changes in the rate of cellular division (e.g. thymidine uptake), changes in the expression of RNA or proteins, changes in cellular localization or modification patterns of RNA or proteins, and changes in the rate of uptake, release or metabolism of nutrients.

Especially potent or weak metastatic genes may be detected by treating and analyzing the metastatic potential of different cells and selecting a suitable cell type as the starting material. For example, cells may be treated with *myc*, *ras*, *p53* or combinations thereof and analyzed for *cyclin D1* expression which is shown to correlates with metastasis. Figure 2 shows the *in situ* analysis of *cyclin D1* in primary MPR tumors (Figure 2A) and in metastatic deposits from the lung of the same animal (Figure 2B). The gene expression pattern of *cyclin D1* in MPR correlates with that of human prostate tumors (Figure 3) analyzed with stains specific for *cyclin D1* expression. Normal human tissue shows no *cyclin D1* expression or staining (Figure 3A). Moderately differentiated prostate cancers with dispersed (Figure 3B) or focal positively staining (Figure 3C) show moderate staining. Advanced poorly differentiated prostate cancer show strong nuclear as well as cytoplasmic staining (Figure 3D) implying strong expression of *cyclin D1*. After treatment with *myc*, *ras* or *p53*, *cyclin D1* expression shows correlation with the metastatic potential of the cell. Thus, *cyclin D1* expressing cells are a source of cells with high metastatic potential. Conversely, cells with low *cyclin D1* expression are a source of potentially metastatically resistant cells.

This method may be adjusted for the isolation of metastatic sequences expressed along a particular developmental or differentiation pathway by combining the various treatment and analytical techniques. This approach is schematically represented in Figure 4. For example, a mammalian cell may be genetically ablated for *TGF- β 1*, *Cyclin D1*, *p53*, *lysyl oxidase*, *caveolin*, *actin binding protein*, *ubiquitin activating enzyme*

E1, *nmb*, α *actinin 3*, or *p34*. The genetically altered cell is used in a *in vivo* mouse prostate reconstitution (MPR) model. Metastatic and nonmetastatic cells isolated from the MPR may be analyzed directly or after induction with an agent such as the *TGF- β* gene or its product. Analysis involves the use of differential display polymerase chain reaction to identify differentially expressed bands. Sequences identified may be used for subsequent ablation, transformation or differential analysis.

Genetic ablation (gene knockout) may be performed after a cell is selected or by selecting a cell comprising a genotype with the proper genetic ablation. Cells already comprising gene ablation may be acquired from a cell depository, from other laboratories or from a transgenic animal. As transgenic animals comprise genetically ablated genes in every cell, any tissue from a transgenic animal may be used as the starting material.

The effects of oncogenes are at least additive and often synergistic. Thus, dominant oncogenes may be transfected together or multiple recessive oncogenes ablated together for a stronger effect. Furthermore, both methods may be combined and dominant oncogene transfection may be accompanied by recessive oncogene ablation.

The function of the metastatic sequence may be determined by the differential expression pattern. For example, a dominant metastatic gene will be present in a metastatic cell while a recessive metastatic gene is present in a non-metastatic cell. Metastatic sequences may be detected as bands which are present in the DD-PCR of metastases isolated in secondary sites and absent from DD-PCR products of primary cells. These sequences may be dominant metastatic genes whose expression is directly responsible for metastases, or they may be metastasis associated genes whose expression correlates with metastasis. Either are useful for therapy and diagnosis. Conversely, DD-PCR bands which are present in primary site tumors, but absent in secondary metastatic sites, may be dominant metastasis suppression genes. Dominant metastasis suppression genes comprise genes

whose expression suppresses metastasis while nonmetastatic genes comprise genes whose expression correlates with non-metastatic tissue. Genes which are highly correlative with either the metastatic phenotype or the non-metastatic phenotype may be isolated. Isolation can be performed by cutting
5 the appropriate nucleic acid in the band of a polyacrylamide gel or by collecting the appropriate fraction in an HPLC or capillary electrophoresis. The nucleic acid may be cloned into a plasmid vector, and sequenced, or synthetically prepared.

Another embodiment of the invention is directed to a method
10 for identifying sequences in a metastatic pathway which are responsive or unresponsive to extracellular signals. Such sequences may be used in therapy and diagnosis of metastatic disorders. Implanted cells or cells from a primary site and cells from a secondary site are treated with extracellular signals. RNA sequences from the treated cells are compared with RNA
15 sequences of the untreated cells (Figure 5B). Treated cells and untreated cells may be derived from a short term or long term *in vitro* culture of primary tumor and malignant tumors. Alternatively, a part of a primary tumor and a part of a malignant tumor may be collected before the animal is treated with an extracellular cytokine or other factor. Long term cultures, or
20 cell lines of primary and malignant cells may also be used as recipients of extracellular growth signal treatment. Suitable signals for each experiment will depend on the cell type. Generally, growth factors, lymphokines, inhibitory factors, migratory factors or hormones may be used. Factors previously isolated by commercial or methods of the invention and factors
25 associated with or causative or suppressive of metastasis are preferred. Thus, transforming growth factor $\beta 1$ (TGF- $\beta 1$) may be used to treat cells before DD-PCR analysis. Proteins encoded by the genes isolated by this method are especially useful for the treatment of cells for the isolation of additional sequences. The identification of one sequence responsive to the

extracellular signal pathway allows for identification of additional genes upstream and downstream from that sequence.

Another embodiment of the invention is directed to metastatic sequences identified by the methods of the invention. Metastatic sequences
5 are sequences associated with the presence or absence of a metastasis or related to the metastatic processor can be used in the therapeutic treatment of metastasis. Metastatic-related sequences include dominant metastatic sequences, recessive metastatic sequences, metastasis associated sequences, dominant oncogenes, recessive oncogenes and cell cycle genes. These genes
10 encode for example, proteins involved in cell cycle, signal processing, DNA replication, growth regulation, inter and intra cellular signaling transcription control and translation control. Isolated sequences are useful in the treatment and for the detection of metastatic and other disorders. Disorders which may be treated comprise diseases involving proteins and sequences
15 which are isolated by interaction with the sequences and proteins isolated by the method of the invention. Both malignant or nonmalignant disorders may be treated. Non malignant disorders include hyperplasia, dysplasia and hypertrophy. Examples of nonmalignant disorders include benign enlargement of the prostate, nodular hyperplasia, and benign prostatic
20 hypertrophy.

Treatment may involve gene replacement, gene targeting, antisense inhibition, gene expression or gene suppression. Gene replacement involves replacing a copy of a defective gene with another copy by homologous recombination. Gene targeting involves the disruption of a
25 cellular copy of a gene by homologous recombination. Antisense inhibition exploits the specificity of hybridization reactions between two complementary nucleic acid chains to suppress gene expression. Cloned genes can be engineered to express RNA from only one or the other DNA strands. The resultant RNA hybridizes to the sense RNA and inhibit gene
30 expression. Gene expression and gene suppression involve the introduction

of genes whose expression actively inhibits neoplastic transformation and metastasis.

Another embodiment of the invention is directed to nucleic acids which comprise a sequence identified by the methods of the invention.

- 5 The nucleic acid may be DNA, RNA or PNA and may be used as a diagnostic tool in the treatment of neoplastic disorders and malignant tumors. The nucleic acids may comprise additional sequences such as promoters, for expression of a sense or antisense message, recombination sequences for gene targeting, selectable markers for transfections, or replication origins for
- 10 passage in a prokaryotic or eukaryotic host such as animal cells, bacteria or yeast.

- Another embodiment of the invention is directed to nucleic acids which comprise sequences identified by the method of the invention such as, for example, the caveolin, ABP280 (actin binding protein 280), the
- 15 lysyl oxidase gene, and the nmb gene (clone 29), and other sequences listed in Figure 12 and Figure 13. Nucleic acids comprising a sequence corresponding to these genes may be used in treatment or diagnosis and in diagnostic kits for screening biological samples for the presence or absence of metastasis or metastatic potential. Treatment may involve using the
- 20 sequences in gene therapy, including gene ablation, gene expression and antisense suppression. Diagnosis may involve genotypic analysis of samples to determine the existence and expression levels of the expressed sequences.

- Another embodiment of the invention is directed to the use of caveolin gene and protein in the isolation of oncogenes and in the treatment
- 25 of neoplastic disorders such as, for example, prostate cancer. Caveolin is an integral membrane protein and a principal component of caveolae. Caveolae are small invaginations at or near the plasma membrane of most smooth muscle cells and may function as a component of specific signal transduction pathways. Surprisingly, caveolin expression increases in metastatic human
- 30 prostate cells as compared to human primary prostate tumors.

As caveolin expression correlates with metastasis, application of biological technologies designed to block the activity of caveolin or the function of caveolae may have therapeutic benefits for the treatment of neoplastic disorders such as human prostate tumors. Specific treatment approaches using caveolin may include the delivery of antisense or dominant negative caveolin sequences using expression or viral vectors; as well as the use of specific anti-caveolin antibodies. Additional approaches could also target the caveolae, but are not specifically based on caveolin function. Additional protein and non-protein components of caveolae could also be targeted for abrogation or the local or systemic administration of nutritional or biological agent may also be used. For example, caveolae are extremely rich in cholesterol and disruption or depletion of this molecule may alter the function of caveolae.

Another embodiment of the invention is directed to methods for treating a neoplastic disorder comprising administering a pharmaceutically effective amount of composition containing a nucleic acid having a sequence identified according to the methods of this invention, its expression product or fragments of either. The nucleic acid may be in the form of a sense or antisense single-stranded or double-stranded nucleic acid. The composition may be combined with a pharmaceutically acceptable carrier such as water, alcohols, salts, oils, fatty acids, saccharides, polysaccharides administered by injection, pulmonary absorption, topical application or delayed release. More than one carrier may be used together to create a pharmaceutical with desirable properties.

Another embodiment of the invention is directed to a kit or diagnostic acid for screening biological samples for detection of metastasis, neoplasia or kits comprise sequences isolated according to the methods of the invention and reagents and materials useful in such kits, such as, for example, buffers, salts, preservatives, and carriers, all of which are well known to those of ordinary skill in the art. Kits are useful for the analysis

of tissues to screen those for the determination of normal, nonmalignant neoplastic or malignant cells. Kits may comprise additional reagents useful for the extraction of nucleic acids from a tissue sample. Reagents for analyzing the nucleic acid extracted from a tissue sample such as polymerase chain reaction reagents and Southern blots reagents may also be included.

The following experiments are offered to illustrate embodiments of the invention and should not be viewed as limiting the scope of the invention.

Examples

10 Example 1 Production of Mouse Prostate Reconstitution Tumors and Metastasis.

Mouse Urogenital Sinus (UGS) tissue was isolated from 17 day old mice embryos. Each isolated UGS was digested with 1% trypsin for three hours at 4°C. The trypsin was inactivated by the addition of fetal calf serum. UGS cells were digested with 0.125% collagenase for 1.5 hours, counted and mixed at the appropriate cell ratios prior to infection with retrovirus in the presence of polybrene. Retroviruses used include Zipras/myc-9. Control experiments were performed using BAG α virus. After a two-hour infection, the infected cells were centrifuged and individual reconstitutions containing 1.5×10^6 cells produced by resuspending the cells in rat tail collagen at a density of 6.0×10^7 cells per ml. Aliquots of the infected UGS cells were placed in (DME) with 10% fetal calf serum overnight at 37°C, 5% CO₂.

The next morning each cell/collagen reconstitution was implanted under the renal capsule of an adult male +/- animal. Reconstitutions were harvested from the mice five weeks later when they showed signs of obvious distress from the tumor burden. Metastasized tumors were isolated from the same mice at sites outside the renal capsule.

Isolated tumors and metastasises were either stored in liquid nitrogen or in preservatives such as 10% buffered formalin.

Cell lines were derived from fresh tumors by mincing a small portion of the primary metastatic or nonmetastatic tumor and placing each
5 in explant culture in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum. Cells which grow from each explant were propagated in DMEM and 10% fetal calf serum.

For histological analysis, a portion of a fresh tumor was fixed in 10% buffered formalin and embedded in paraffin for sectioning and
10 staining with hematoxylin and eosin (H&E) or immunohistochemical staining. Immunohistochemical localization of cytokeratins was detected using polyclonal cytokeratin antiserum A575 (Dake Co.; Carpinteria, CA) and Vectastain ABC kit (Vector Laboratories; Burlingame, CA).

Example 2 Isolation of C-DNA for DD-PCR.

15 Total cellular RNA was isolated by ultracentrifugation through cesium chloride. Briefly, up to one gram of cells from culture, tumors or organs was placed into 4 ml of ice-cold GIT buffer (4M guanidine isothiocyanate, 0.025 M sodium acetate, 0.1 M β -mercaptoethanol) and homogenized in a tissue homogenizer (Polytron or equivalent). The
20 homogenate was carefully layered over 4 ml of 5.7 M CsCl, 0.024 M sodium acetate (1.8 g CsCl per ml) in a centrifuge tube. The layers were centrifuged at 35,000 RPM for 18 hours in a SW50.1 rotor. DNA was collected from the interface between the cushion and the supernatant, diluted two folds with water, added to 2.5 volumes of ethanol and spooled out on a glass rod. RNA
25 that formed a pellet on the bottom of the CsCl layer was resuspended, and once extracted with an equal volume of phenol:chloroform (1:1), twice with chloroform and precipitated with ethanol and resuspended in diethylpyrocarbonate treated water. The concentration of DNA and RNA were be determined by absorption at 260 nanometers.

Example 3 Differential Display Polymerase Chain Reaction.

mRNA isolated from primary tumors or metastasis was reverse transcribed with one of the primers and subjected to DD-PCR using the same primer as both the forward and reverse primer. A set of 24 primers
 5 comprising short oligonucleotides were used for both the reverse transcription of mRNA into c-DNA and for differential display polymerase chain reaction. The sequence of the primers used are shown in Table 1.

Table 1

Primer No.	Sequence	Sequence number
1	5'-TGACAATCG-3'	(SEQ. ID. NO. 1)
2	5'-AGCTAAGGTC-3'	(SEQ. ID. NO. 2)
3	5'-TCTGCGATCC-3"	(SEQ. ID. NO. 3)
4	5'-ATACCGTTGC-3'	(SEQ. ID. NO. 4)
5	5'-TACGAAGGTG-3'	(SEQ. ID. NO. 5)
6	5'-TGGATTGGTC-3'	(SEQ. ID. NO. 6)
7	5'-CTTTCTACCC-3'	(SEQ. ID. NO. 7)
8	5'-GGAACCAATC-3'	(SEQ. ID. NO. 8)
9	5'-TGGTAAAGGG-3'	(SEQ. ID. NO. 9)
10	5'-TCGGTCATAG-3'	(SEQ. ID. NO. 10)
11	5'-CTGCTTGATG-3'	(SEQ. ID. NO. 11)
12	5'-GATCAAGTCC-3'	(SEQ. ID. NO. 12)
13	5'-GATCCAGTAC-3'	(SEQ. ID. NO. 13)
14	5'-GATCACGTAC-3'	(SEQ. ID. NO. 14)
15	5'-GATCTGACAC-3'	(SEQ. ID. NO. 15)
16	5'-TTAGCACCTC-3'	(SEQ. ID. NO. 16)
17	5'-ACCTGCATGC-3'	(SEQ. ID. NO. 17)
18	5'-GCTATACTGC-3'	(SEQ. ID. NO. 18)
19	5'-AGTTGCCAGG-3'	(SEQ. ID. NO. 19)

20	5'-AAGCCGTGTC-3'	(SEQ. ID. NO. 20)
21	5'-TCAACGCTCA-3'	(SEQ. ID. NO. 21)
22	5'-TGTTCGAATC-3'	(SEQ. ID. NO. 22)
23	5'-CGAGTCAGAC-3'	(SEQ. ID. NO. 23)
24	5'-TATGAGTCCG-3'	(SEQ. ID. NO. 24)

PCR was performed using standard conditions with 40 cycles of denaturation at 94°C for 40 seconds, annealing at 40°C for 2 minutes, and elongation at 72°C for 35 seconds. After PCR, the products were analyzed with non-denaturing polyacrylamide gel electrophoresis (PAGE) at 12 watts for 15 hours. Bands which differed between test and control samples were eluted from the gel, subjected to reamplification by PCR and cloned. Polyacrylamide gel electrophoresis of DD-PCRs, and the accompanying RNA blot analysis showing the isolation of sequences with substantial similarity to *nmb* and TGF- β is shown in Figure 6 and Figure 7 respectively. Additional sequences isolated by this method show substantial similarity to lysyl oxidase, actin binding protein, ubiquitin activating enzyme E1, α -actinin, and P34 ribosomal binding protein sequence (Figure 8). Differential expression of caveolin was demonstrated by DD-PCR followed by PAGE (Figure 9).

Example 4 p53 Allelotype Determination.

The *p53* allelotype of a cell sample was determined by PCR. Briefly, nucleic acid is extracted from a tissue sample or a cell culture sample. An aliquot of nucleic acids is placed in 45 μ l aliquot of a master mix which contained a final concentration of 0.2 mM of each dATP, dTTP, dGTP, dCTP, 1.5 mM MgCl₂, 0.5 unit Taq polymerase, 0.05 μ M of each of two primers set specific for the normal wildtype allele of *p53* (5'-GTGTTTCATTAGTTCCCCACCTTGAC-3', SEQ. ID. NO. 25; 5'-

AGAGCAAGAATAAGTCAGAAGCCG-3', SEQ. ID NO. 26). A control set of primers specific for the fibroblast growth factor-7 gene was used to monitor the polymerase chain reaction experiment (5'-ACAGACCGTGCTTCCACCTCGTC-3', SEQ. ID NO. 27; 5'-CCTCATCTCCTGGGTCCCTTTCA-3', SEQ. ID NO. 28). One μ l of the reaction from the first round of PCR was used as the starting material for a second round of PCR using a second set of wildtype *p53* specific primer (5'-GTCCGCGCCATGGCCATATA-3', SEQ. ID NO. 29; 5'-ATGGGAGGCTGCCAGTCCTAACCC-3', SEQ. ID NO. 30). This second round of PCR was also monitored using a control set of primers specific for the fibroblast growth factor-7 (5'-ACAGACCGTGCTTCCACCTCGTC-3', SEQ. ID NO 27; 5'-CCTCATCTCCTGGGTCCCTTTCA-3', SEQ. ID NO 28).

After PCR the products were analyzed with non-denaturing polyacrylamide gel electrophoresis (PAGE) at 12 watts for 15 hours. Bands which differed between test and control were eluted from the gel, subjected to reamplification by PCR and cloned.

Example 5 Induction of cell lines with *TGF- β 1* Influence Cellular Gene Expression.

1481-PA cells were grown overnight in DME supplemented with 10% fetal calf serum overnight at 37°C, and 5% CO₂. Induction was performed by treatment with *TGF- β 1* at a concentration of 2 nanograms per ml. The treated cells were returned to the incubator and cultured for 12 hours. After induction, cells were washed in phosphate buffered saline and harvested and concentrated by centrifugation.

RNA was extracted from treated and untreated cells and subjected to DD-PCR. Differentially expressed bands detected by DD-PCR were cloned and differential expressions were confirmed using RNA blots

(Figure 10). Subsequent cloning and sequencing identified the bands as ABP280 or filamin.

One gene isolated showed differential expression in cells induced by *TGF- β* (Figure 11, clone 29), while a control probe on the same cell line showed no difference in expression levels (Figure 11, GAPDH).

Example 6 Metastatic Sequences Isolated.

Using the methods of Examples 1, 2, 3, 4, and 5, a plurality of metastatic sequences were isolated and sequenced. The expression of the metastatic sequences in primary cells and in metastatic cells were determined using RNA blots. The nucleic acid sequences of other isolated sequences are listed in Figure 12. Sequence analysis and expression analysis was performed on the isolated cloned and the results of these studies are summarized in Figure 13.

Example 7 Caveolin Immunoassay in Human Prostate Cancers.

Primary site human prostate tumors and metastases were isolated and analyzed for caveolin expression by immunoassay. The results of the assay is shown in Table 3. Metastases shows higher levels of caveolin proteins in metastases than in primary tumors. Immunohistology of tissue sections reveals both elevated levels and distinct distribution of caveolin protein in metastatic human prostate when compared to a primary human prostate tumor (Figure 14).

Table 3

Patients	Primary-site	Metastases in lymph node
1	+	++
2	++	+++

34

5

10

3	++	+++
4	++	++
5	+	+
6	++	++
7	++	+++
8	+	+
9	-	-
10	+	+
11	+	+
12	++	++
13	+	+
14	++	+++

Other embodiments and uses of the invention will be apparent
15 to those skilled in the art from consideration of the specification and practice
of the invention disclosed herein. The specification and examples should be
considered exemplary only with the true scope and spirit of the invention
indicated by the following claims.

I Claim:

1. A method for identifying a metastatic sequence comprising the steps of:
 - a) transfecting an oncogenic sequence into a mammalian cell to
5 form a population of transfected cells;
 - b) introducing transfected cells to a primary site of a host mammal;
 - c) maintaining said mammal for a period of time sufficient to develop a metastasis at a secondary site;
 - 10 d) amplifying expressed sequences of the transfected cells and expressed sequences of the metastasis by differential-display PCR; and
 - e) comparing the amplified sequences and identifying the metastatic sequence.
- 15 2. The method of claim 1 wherein the mammalian cell is transfected by calcium phosphate transfection, viral transduction, lipofection, dextran sulfate transfection or electroporation.
3. The method of claim 1 wherein the oncogenic sequence is a sequence of the gene that expresses the oncoproteins p21, p34, p53, myc, ras or src.
- 20 4. The method of claim 1 wherein the oncogenic sequence is a metastatic sequence.
5. The method of claim 4 wherein the metastatic sequence is a sequence of the gene that expresses cyclin D1, caveolin or TGF- β 1.
6. The method of claim 1 wherein the oncogenic sequence is a gene
25 ablation sequence specific for the gene that expresses the protein TGF- β 1, cyclin D1, p21, p34, p53, lysyl oxidase, caveolin, actin binding protein, ubiquitin activating enzyme E1, nmb or α actinin 3.
7. The method of claim 1 wherein the mammalian cell is treated with a metastatic agent that alters gene expression before or after transfection.

8. The method of claim 8 wherein the metastatic agent is benzanthrane (BA), dimethyl benzanthrane (DMBA) or 5-azacytidine.
9. The method of claim 1 wherein the mammalian cell is a primary or established cell line.
- 5 10. The method of claim 1 wherein the mammalian cell is derived from urogenital sinus tissue.
11. The method of claim 1 wherein the mammalian cell is a fetal cell.
12. The method of claim 1 wherein the mammalian cell contains a genetically ablated endogenous gene wherein said gene is *TGF- β 1*, *cyclin*
10 *D1*, *p21*, *p34*, *p53*, *ras*, *myc* and homologs thereof.
13. The method of claim 1 wherein the mammalian cell is derived from the same species as the host mammal.
14. The method of claim 1 wherein the mammalian cell and the host mammal are histocompatible.
- 15 15. The method of claim 1 wherein the mammalian cell and the host mammal are genetically matched.
16. The method of claim 1 wherein the transfected cell is maintained *in vivo* or *in vitro*.
17. The method of claim 1 wherein a collection of the expressed
20 sequences is obtained from cells at the primary site of the host mammal.
18. The method of claim 1 wherein a collection of the expressed sequences is obtained from a cell line of immortalized transfected cells.
19. The method of claim 1 wherein the transfected cells are introduced to the primary site by subcutaneous implantation.
- 25 20. The method of claim 1 wherein the host mammal is a mouse, a rabbit or a primate.
21. The method of claim 1 wherein the host mammal is an syngeneic, xenogeneic, immunocompromised or transgenic host mammal.
22. The method of claim 1 wherein the host mammal has reduced
30 expression of *TGF- β* .

23. The method of claim 1 wherein the primary site is the renal capsule, the prostate or the testis.
24. The method of claim 1 wherein the secondary site is selected from the group of sites consisting of lung, kidney, liver, lymph nodes, brain, bone, 5 testis, spleen, ovaries and mammary.
25. The method of claim 1 wherein differential display PCR is performed with an anchor primer and a variable primer.
26. The method of claim 25 wherein the anchor primer comprises a polythymidine sequence and a dinucleotide sequence connected to a 3'- 10 terminus.
27. The method of claim 26 wherein the polythymidine sequence comprises between about 5 to about 30 thymidines.
28. The method of claim 26 wherein the dinucleotide sequence is selected from the group of sequences consisting of AA, AG, AC, AT, GA, GG, GC, 15 GT, CA, CG, CC and CT.
29. The method of claim 25 wherein the anchor primer or the variable primer comprise a detectable moiety selected from the group consisting of radioactive moieties, phosphorescent moieties, magnetic moieties, luminescent moieties and conjugatable moieties.
- 20 30. The method of claim 25 wherein the anchor primer and the variable primer have a common sequence.
31. The method of claim 1 further comprising the step of treating the host mammal with a metastatic agent.
32. The method of claim 31 wherein the metastatic agent is a retinoid.
- 25 33. The method of claim 1 wherein identifying comprises determining the nucleotide sequence or expression product of the metastatic sequence.
34. The method of claim 1 wherein the metastatic sequence identified is specifically expressed in metastatic or non-metastatic cells.
35. A metastatic sequence identified by the method of claim 1.

36. The metastatic sequence of claim 35 which is a sequence which encodes *TGF- β 1*, *Cyclin D1*, *lysyl oxidase*, *caveolin*, *actin binding protein*, *ubiquitin activating enzyme E1*, *nmb*, *α -actinin 3* or homologs thereof.

37. A method for identifying a metastatic sequence comprising the steps
5 of:

- a) pretreating a mammalian cell with a metastatic agent to form a population of cells predisposed to metastasis;
- b) introducing the pretreated cells to a primary site of a host mammal;
- 10 c) maintaining said mammal for a period of time sufficient to develop a metastasis at a secondary site;
- d) treating cells of the primary or secondary sites with a genotoxic agent;
- e) amplifying expressed sequences of treated cells by
15 differential-display PCR; and
- f) identifying the metastatic sequence.

38. The method of claim 37 wherein the metastatic agent is an oncogenic sequence and the mammalian cell is treated by transfection with the oncogenic sequence.

20 39. The method of claim 37 wherein the metastatic agent is *TGF- β 1*, *Cyclin D1*, *p21*, *p34*, *p53*, *lysyl oxidase*, *caveolin*, *actin binding protein*, *ubiquitin activating enzyme E1*, *nmb* or *α -actinin 3*, and the mammalian cell is treated by contact with the metastatic agent.

40. The method of claim 37 wherein the genotoxic agent is
25 benzanthrane (BA), dimethyl benzanthrane (DMBA) or 5-azacytidine.

41. The method of claim 37 wherein the metastatic agent and the genotoxic agent are the same.

42. The method of claim 37 wherein the expressed sequences amplified are compared to expressed sequences amplified from mammalian cells
30 before pretreatment to identify the metastatic sequence.

43. The method of claim 37 wherein the expressed sequences amplified are compared to expressed sequences amplified from pretreated cells to identify the metastatic sequence.
44. The method of claim 37 wherein the expressed sequences amplified
5 are compared to expressed sequences amplified from cells obtained from the primary site or cells obtained from the secondary site.
45. A nucleic acid sequence identified by the method of claim 37.
46. A method for identifying a metastatic sequence comprising the steps of:
- 10 a) treating a mammalian cell with a metastasizing agent to form a population of treated cells;
- b) introducing treated cells to a primary site of a host mammal;
- c) maintaining said mammal for a period of time sufficient to develop a metastasis at a secondary site;
- 15 d) amplifying RNA sequences of treated cells and RNA sequences of the metastasis by differential-display PCR;
- e) comparing the amplified sequences and identifying the metastatic sequence.
47. The method of claim 46 wherein the metastatic agent is a chemical
20 compound, a nucleic acid, a protein or a combination thereof.
48. The method of claim 47 wherein the chemical compound is a benzanthrane, dimethyl benzanthrane, or 5-azacytidine.
49. The method of claim 47 wherein the nucleic acid contains an oncogenic sequence.
- 25 50. The method of claim 47 wherein the protein is p53, myc, ras, caveolin or TGF- β 1.
51. The method of claim 46 wherein the mammalian cell is transfected with an oncogenic sequence before or after treatment.
52. The method of claim 46 wherein the mammalian cell is a cell line.

53. The method of claim 46 wherein the mammalian cell is derived from lymphatic tissue, hematopoietic cells, reproductive tissues or urogenital sinus tissue.
54. The method of claim 46 wherein the mammalian cell is a fetal cell.
- 5 55. The method of claim 46 wherein the mammalian cell is derived from a transgenic animal.
56. The method of claim 46 wherein the primary site is the renal capsule, the prostate or the testis.
57. The method of claim 46 wherein the secondary site is selected from
10 the group of sites consisting of lung, kidney, liver, lymph nodes, brain, bone, testis, spleen, ovaries and mammary.
58. The method of claim 46 wherein differential display PCR is performed using an anchor primer and a variable primer.
59. A metastatic sequence identified by the method of claim 46.
- 15 60. A diagnostic kit for screening a biological sample for the presence or absence of metastasis comprising a metastatic sequence identified according to the method of claim 46.
61. A method for treating a metastatic disorder comprising administering a composition containing a therapeutically effective amount of a metastatic
20 sequence or the expression product of said metastatic sequence to a patient wherein said metastatic sequence was identified according to the method of claim 46.
62. The method of claim 61 wherein said metastatic sequence is selected from the group consisting of *TGF- β 1*, *Cyclin D1*, *p21*, *p34*, *p53*, *lysyl oxidase*, *caveolin*, *actin binding protein*, *ubiquitin activating enzyme E1*,
25 *nmb*, *α actinin 3* and homologs thereof.

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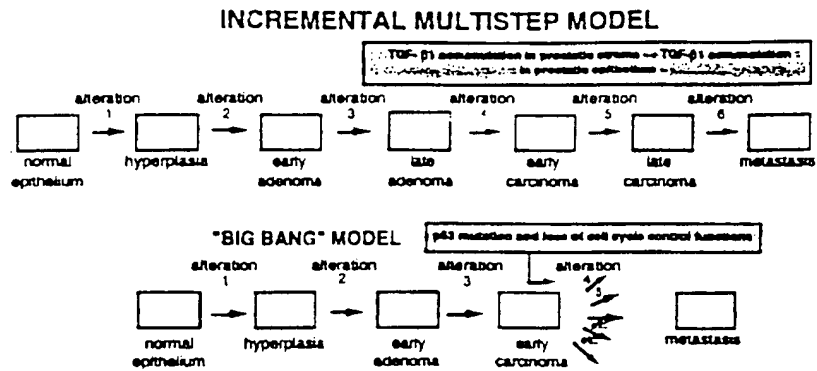


FIGURE 1

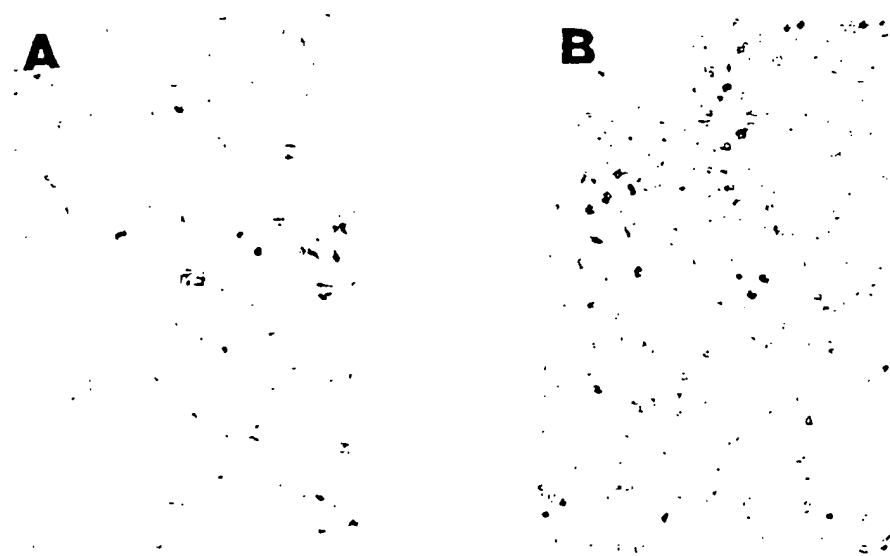


FIGURE 2

FIGURE 3A



FIGURE 3B

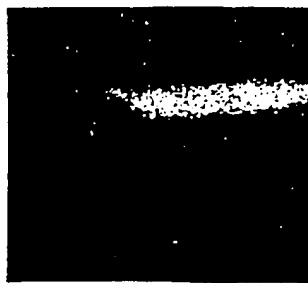


FIGURE 3C



FIGURE 3D

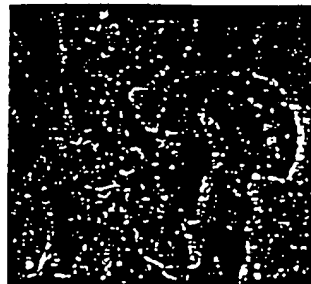


FIGURE 3

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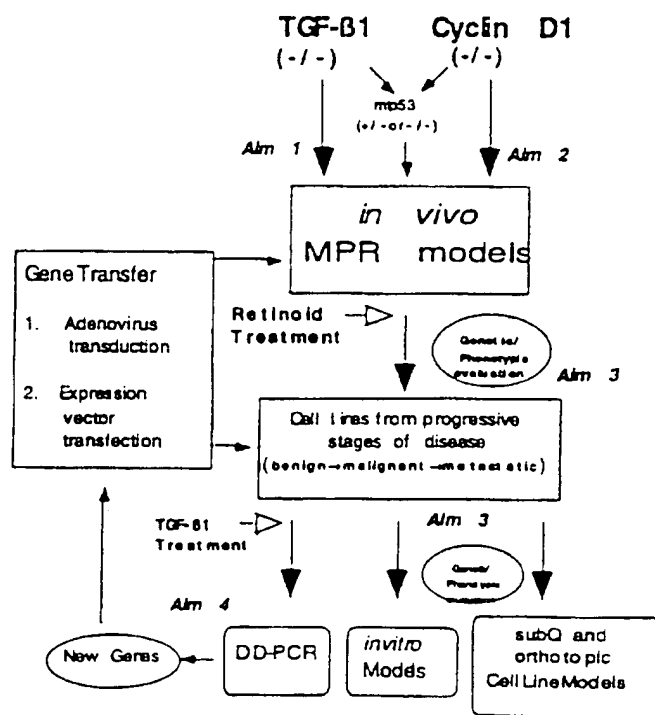


FIGURE 4

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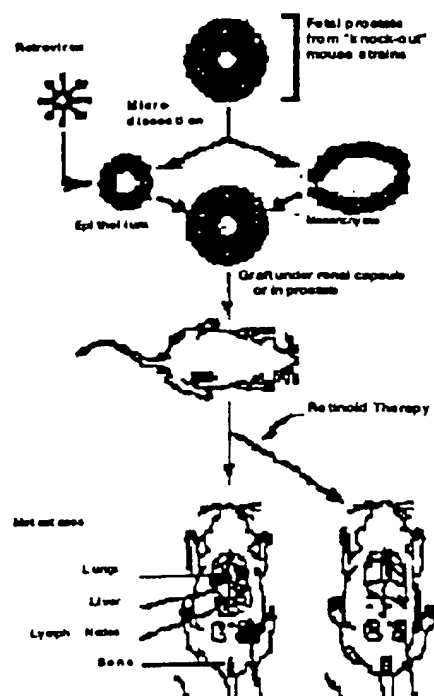


FIGURE 5A

GENES RELATED TO PROSTATE CANCER METASTASIS

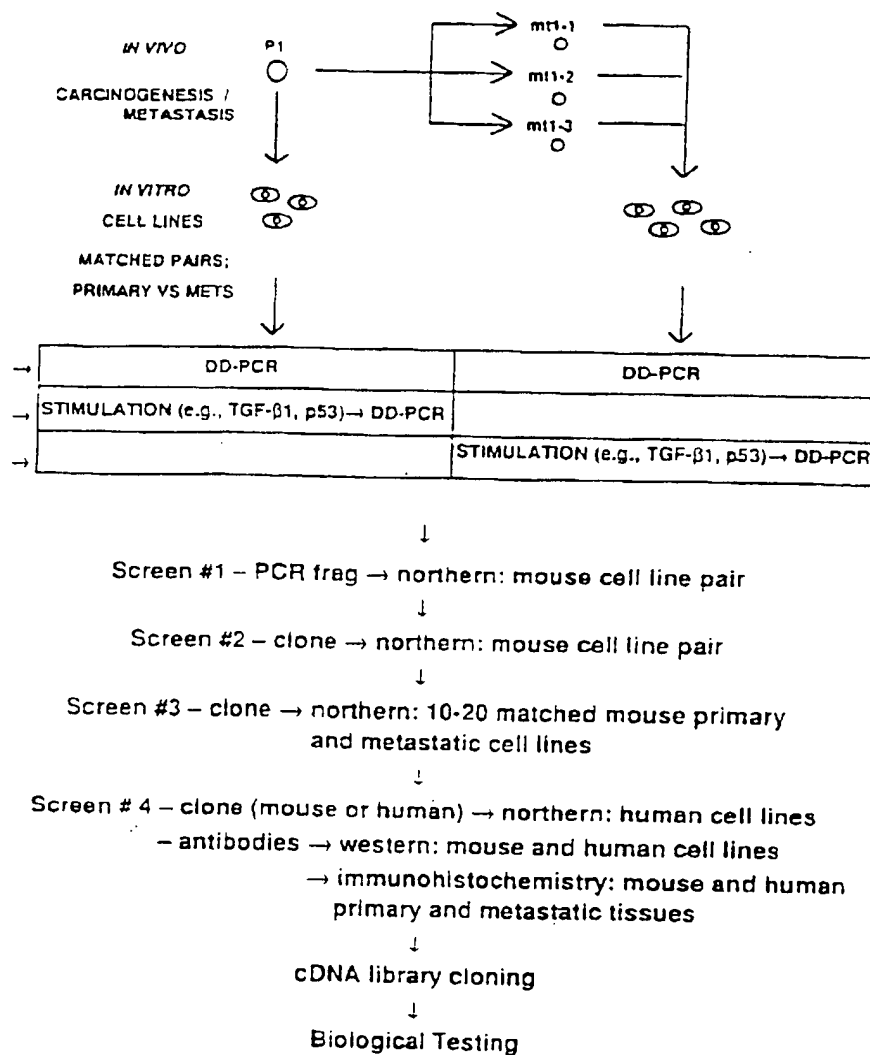


FIGURE 5B

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DD-PCR identification of nmb

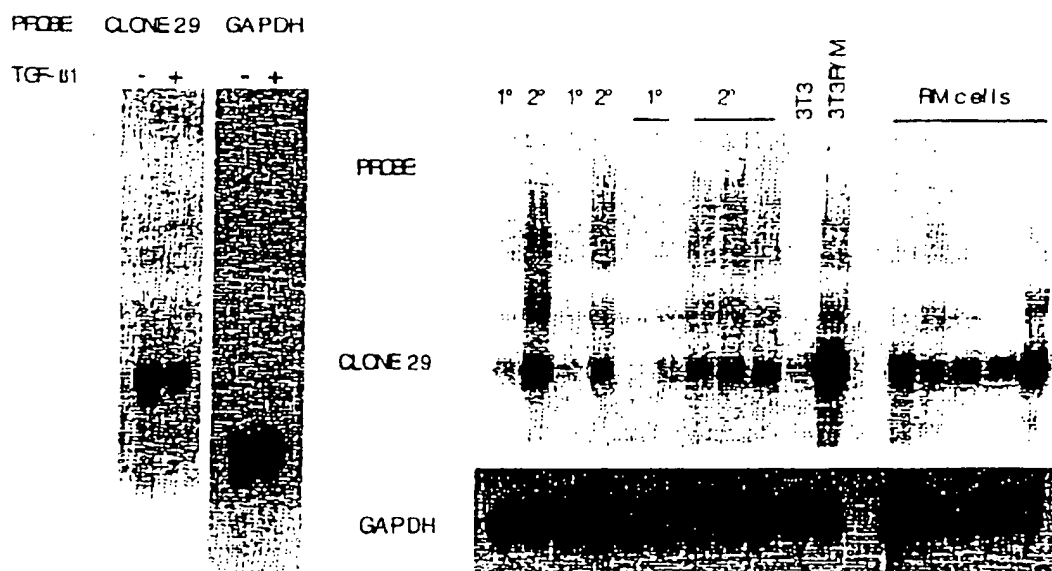


FIGURE 6

FIGURE 7

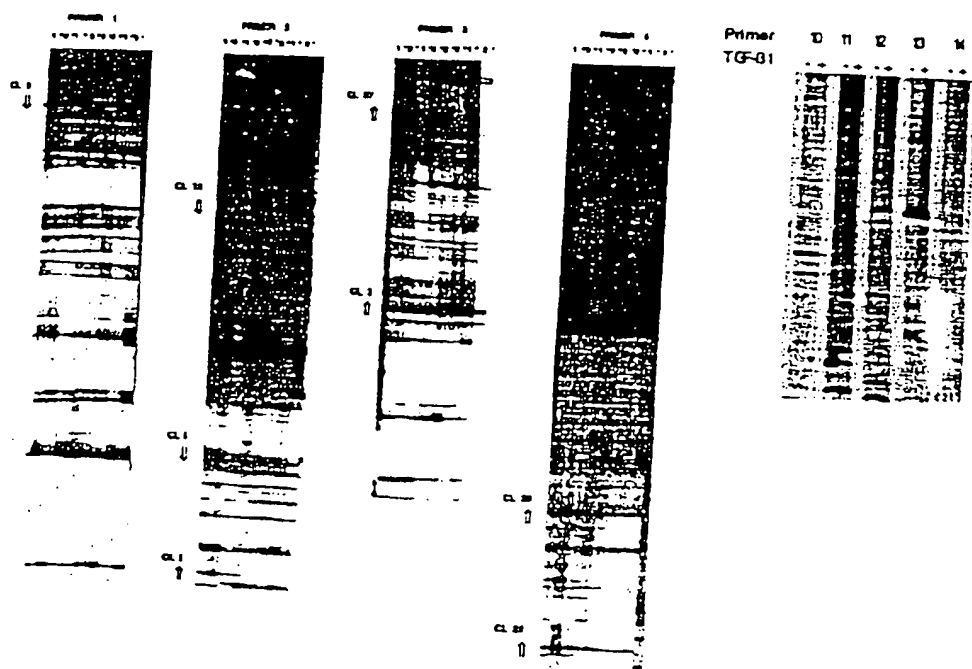


FIGURE 8

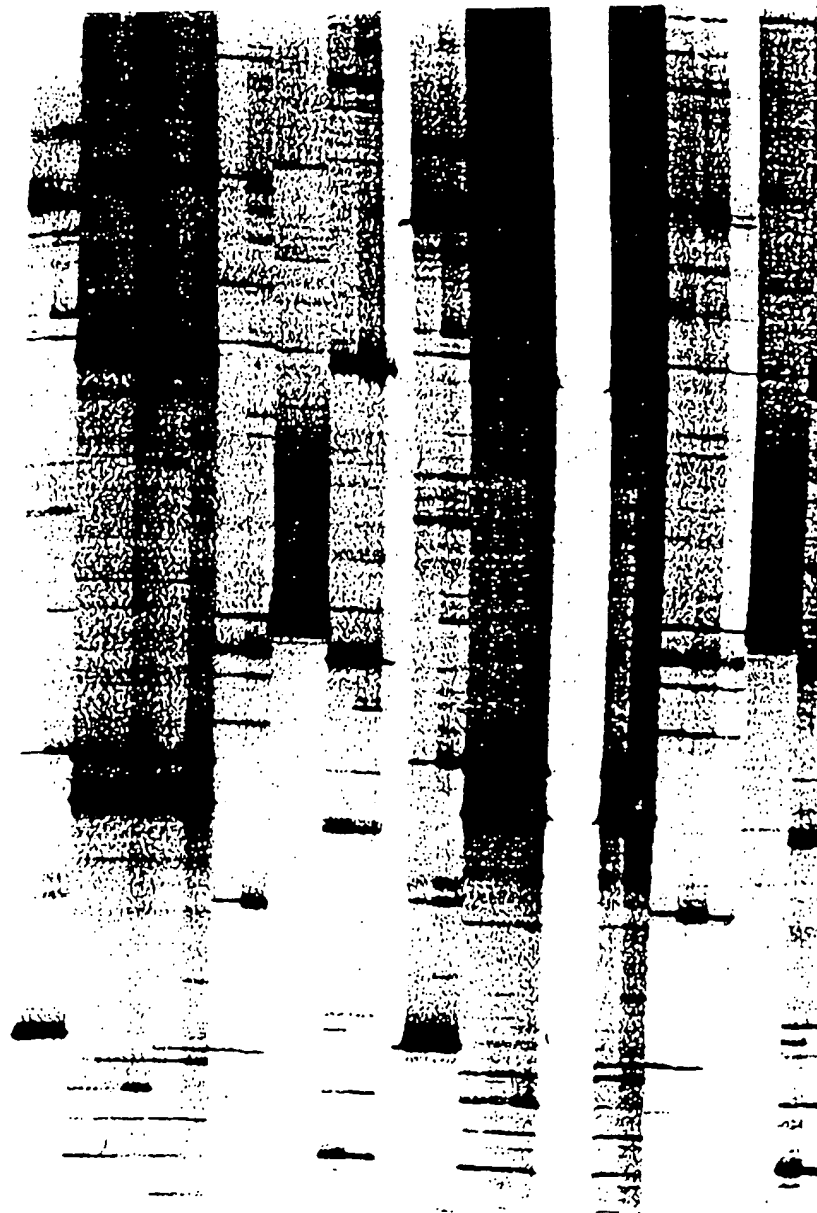


FIGURE 9

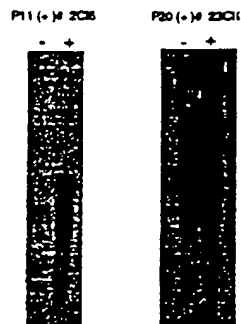


FIGURE 10

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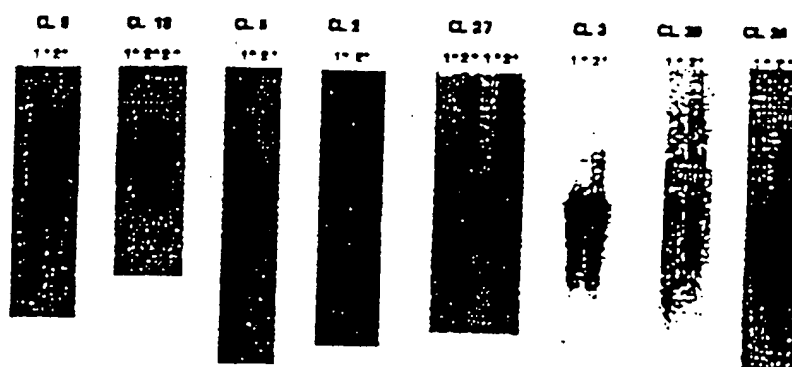


FIGURE 11

FIGURE 12AA CL-1#2

AATTTTTTTTTTCGACGGCCCAACGGAATTTTTTTTTTCGACGGCCCAACGGAATTTT
TTTTTCGACGGCCCAACGGAATTCGGCTTAGCTAAGGTCACCCAGACTTCATGGACT
TGTCTATTTTCTTGCCCAAAGGGATAGTTCCTCAGGTATTTGGGGACAGCATTACCTC
TTGCAGGAGCTATGCCTGTGTGTTTGTGCTAAGTTGATACTTTCTGCGATGATCTCAC

(SEQ ID NO. 31)

FIGURE 12AB CL-10#3

TACCATCGGAGAAAGAAGACCAAGCAAGGCTCAGGCAGCCACCGCCTGCTTCGCACT
GAGCCTCCTGACTCAGACTCAGAGTCCAGCACAGACGAAGAGGAATTTGGAGAATTG
GAAATCGCTCTCGTTTTGTCAAGGGAGACTATCCCGATGCTGCAAGATCTGCTGTCCCT
CTGGCCTTTGTCATCCTCGCGCCTGCGTTGTGGCCTCTGTGGGCTTGGTGTGGAGCAAA
TGGCTCTCAAGGAGGACTGAGTCTCAAGGAAATT

(SEQ ID NO. 32)

FIGURE 12AC CL-11A#5

AGCTAAGGTCAGGAGGTGTCTGAAGAATTGGCTGATGCATGGCAGGGATGTTGTTGAC
CTGCTTTTAGAACAATACTTCCATTTAATTATAGCATACTTATGTGTGTATTAAAGCA
GAGCCGATCTGGTGGGGCTCATTAAGTAAATGTACTTACTGCAAAAGGTTCAACTGGT
GACCCAGTTTTCCCAGAAAGCAATATGATAGGACAGAGGCGACTCCTGCAAGTTGTC
TCAGACTTCACACATACATTGTGACATTCTCTGAGCATGTGCACTGTACATGATATGAC
ACTATCAA

(SEQ ID NO. 33)

FIGURE 12AD CL-11C#2

AGCTAAGGTCCACTACCTTGTGAAGATGTATAAACACCTGAAATGTAGAAGCGATCCG
TATGTCAAGATCGAGGGGAAGGACGCTGACGACTGGCTGTGTGTGGACTTTGGGAGTA
TGGTGATCCATTTGATGCTTCCAGAAACCAGAGAAACCTATGAATTAGAGAACTATG
GACTCTACGTTCTTTTGATGACCTTAGCTAAGCCGAATCAGCACACTGGCGGCGTTACT
AGTGGATCGAGCTCGTACAGCTGATGCATAGCTTGAGTATCTATAGGTTACTAATAGC
TGGCTATCATGTCAAGCGTTC

(SEQ ID NO. 34)

FIGURE 12AE CL-12#1

AGCTAAGGTCAAAATAAAAGCTCAAGATGACATCAGTCCCATTGTCTAAGTCCTGG
TGTTGTATGGATGGTAAGCAGCAGCCAATTATGGTGACAGGTGATAGATCCAATTTGT
TAACATTTCTCCATCTCTAAGCCATCCTTAAAGAAAAATCATGAATGGAGTCACACCAT
CTTCACGGTAGTCCAGGAGAGCAACCATAACCATCTGGATTTCATGTTTCACCAATAAAA
ACTGGTAGTTATTGAATTAGCAAGGATGTGCTACTCTCTGCAGCTCAGC

(SEQ ID NO. 35)

FIGURE 12AF CL-13#1

AGCTAAGGTCTCATGCAATGGAAGTTAATTCTTAGAAGTGAAGAATTACATCAAACA
TAAAAGCCTCCCTATTAAATGTAGTCCACAAAAGTGGCAGGTATATATGCCTTCTGAAT
TTGTCTCCAGTGACTTTGGTAAATCTAACTAAATTTTAAAAATTCTTAATGAATTTAT
CGTCAACAACACACCTCTTGGAAAAATTAACCCTTGCAGTGTCTGTGTTAGACTCAG
AAGTCAA

(SEQ ID NO. 36)

FIGURE 12AG CL-14#4

GAATTCGGCTTAGCTAAGGTCAGCGTGAAGTTTAAGCAGACATGAGTCTGAAACAGTC
TCATGACACATCTGATAGGATTTTTTAAGACTGCCTGGCTTAGTCTTACTGCTGTTAGT
GTATATTAGGTGTTGTACACATTATAAAGAAAATTATGTCTCATTATCTTGTTTAAGTC
AAGGAAAATAGAGAACTTTGGTCAAAT

(SEQ ID NO. 37)

FIGURE 12AH CL-2#2

GAATTCGGCTTAGCTAAGGTCAGCGTGAAGTTTAAGCAGACATGAGTCTGAAACAGTC
TCATGACACATCTGATAGGATTTTTTAAGACTGCCTGGCTTAGTCTTACTGCTGTTAGT
GTATATTAGGTGTTGTACACATTATAAAGAAAATTATGTCTCATTATCTTGTTTAAGTC
AAGGAAAATAGAGAACTT

(SEQ ID NO. 38)

FIGURE 12AI CL-2#3

GAATTCGGCTTAGCTAAGGTCAAAATACACGGATTGCAATCACTTTTCTAAACAAAAG
AAACAAAGTAACTGCTGAGGTTAGCAAAGATGAGTTCTCGTCATACTGCCTTGTAAGT

TTTTGTGAACTGTGTTATTA AAAATCTGAGCTTAACAAAATCTTTACAAGTCACCTCAT
GAAAACAGCATTGGCCAATAAGAGTTTAATTCCACACCAGTGAGACCTTAGCCT

(SEQ ID NO. 39)

FIGURE 12AJ CL-2#4

GAATTCGGCTTTCTGCGATCCACTCTTTGAAGCTATTGGCAAGATATTCAGCAACATCC
GCATCAGCACGCAGAAAGAGATATGAGGGACATTTCAAGGATGAAAGGTTTTTTTCCC
CCCTTACTATTTCCCTTGGTGCCAATTC CAAGTTGCTCTCGCAGCAGCAAATTTATGAAT
GGTTTGTCTTGATCAAGAACAAGAATTCATCCACCAATTCTCATATATACTACTTTCT
TCTTCTT

(SEQ ID NO. 40)

FIGURE 12AK CL-3#1

GAATTCGGCTTTCTGCGATCCACTCTTTGAAGCTATTGGCAAGATATTCAGCAACATCC
GCATCAGCACGCAGAAAGAGATATGAGGGACATTTCAAGGATGAAAGGTTTTTTTCCC
CCCTTACTATTTCCCTTGGTGCCAATTC CAAGTTGCTCTCGCAGCAGCAAATTTATGAAT
GGTTTGTCTTGATCAAGAACAAGAATTCATCCACCAATTCTCATATATCTACGTCTCT
TCTAG

(SEQ ID NO. 41)

FIGURE 12AL CL-4#1

GAATTCGGCTTTCTGCGATCCTAGAGCAGGTAAGTGAAGAAGGCCAGTAAGTTTAAAG
GATGGCCTTGTTGCCTTCTATCAAGTTCTCTGGGACTTTGTAATTTTGATTACTACTATT
GATACATGGTTATGGTCAGAAGGCCTCTTCTCCCTT

(SEQ ID NO. 42)

FIGURE 12AM CL-4#2

AGCTAAGGTCCGGACTCTATGGCATGACCCCAAAAACATTGGCTGGAAAGATTACACT
GCCTACAGGTGGCACCTGATTCACAGGCCTAAGACAGGCTACATGAGAGTCTTAGTGC
ATGAAGGAAAGCAAGTCATGGCTGACTCAGGACCAATTTATGACCAAACCTACGCTG
GTGGACGGCTGGGCTGTTTGTCTTCTCCAAGAGATGGTCTATTCTCGGACCTCAAGTAT
GAGTGCAGAGATGCTAGAGAGCAGGCTCAGTCTCAGCA

(SEQ ID NO. 43)

FIGURE 12AN CL-5A#4

TGACCATCGAGTGCATCAGCCTCATCGGGCTGGCCGTCGGGAAGGAGAAATTCATGCA
GGATGCTTCAGATGTGATGCAGCTATTGTTGAAGACACAGACAGACTTCAATGATATG
GAAGATGACGACCCCCAGATTTCTTACATGATCTCAGCATGGGCCAGGATGTGCAAAA
TCTTGGGAAAGAATTCAGCAGTACCTTCCCGTGGTTATGGGGCCGCTGATGAAGACT
GCTTCAATTAAGTCTGAGTGCCTCTAGACACCAGGACATGAGATATGAGGTA

(SEQ ID NO. 44)

FIGURE 12AO CL-6#2

TGACCATCGTGTAGTTGGTGTGCTTGTGTGCGAAGATGAGGGCCTCCTGGATGAGCTG
GTGCTGCTGCTCCAGCAGGTCCAGGCTGGGCTTGTAGTCCACGATGCTGCGCTCGTAC
TGCTTCAGGTGGCTCAGCTGGTCTTCCAGAGTCCCGTTTCATCTCAATGGAGATGCGCCC
GATCTCCTCCATCTTAGTCTGGATCCACGGCCCCACCATAATTGGCTTGGCTGGCGAACT
GTCGGCGAAGGCTGCAATTGGATTGCT

(SEQ ID NO. 45)

FIGURE 12AP CL-7#4

TGACCATCGAACACCCCAACACTCTCCACTACCTGCCATTTCTTCCAGCCTTA1CCACA
CCACCCCGTTTCTCCTGAAGACTGATTTGCTTAGCAACTGCACTGAGCCAA1CCCTGAA
GACACATGATTATTGGTTGGGCTCCATTAAACAACAAGCCTAGTGCTTGGGAAGGGGG
GTGGGGAGGGGAAGAGACGTGAGAAGCATGTTGGCGTAGACCTTGAGGCATGGATGA
AGCATCTGCCGGCCTGACCTGGTACAGGTGGCATCTGCACTGCAGCAAGGC

(SEQ ID NO. 46)

FIGURE 12AQ CL-8#2

TGACCATCGAAGTGCAAAGGAAATGACTTGATTTCATGAAGTATCTCCAGAAGTAACG
CTTTGTTTTCTGCATCCTGAACTTTATTCCCAGTGAAGAGCTGAAAATCTGGACGCTCA
AAAAATGGAAGCACTTTGGAGAGAGCCCTTAACTCTATCAGGTACAGGAAGTACAAG
TTCCTCAGCCTTCGTGGGCCTTCTCCTTCAGTCAGAATCCATCAAAGGTGCTGGAAGTC
TGTGACATTGTGACCCATTCTTTCAGCCAGTATCTGTAAGATAC

(SEQ ID NO. 47)

FIGURE 12AR CL-9#1

GGGAACGAATGATCTGGAAGTGTGGCTTGTAGACAACCCAAATATCTTAGGTAGGTAA
GAAATTCAGCATCACACTATATAGGAAATACTGTGCGAAACTGACAGTTAACTGTGC
ACAAAGTTCAATGGCTTCAAAATAATGTATAAAGGATAAGAAGAAACCAGTTTACCAT
TTTGGTATTATTTGGTTGCTTTGTATAACTTCAATAATTT

(SEQ ID NO. 48)

FIGURE 12AS CL-54A#2.-SP

GGGAACGAATGATCTGGAAGTGTGGCTTGTAGACAACCCAAATATCTTAGGTAGGTAA
GAAATTCAGCATCACACTATATAGGAAATACTGTGCGAAACTGACAGTTAACTGTGC
ACAAAGTTCAATGGCTTCAAAATAATGTATAAAGGATAAGAAGAAACCAGTTTACCAT
TTTGGTATTATTTGGTTGCTTTGTATAACTTCAATAATTT

(SEQ ID NO. 49)

FIGURE 12AT CL-54A#2.-S0

GACGTAAAGCC

(SEQ ID NO. 50)

FIGURE 12AU

CCACAAAGCAAGCTTCTGTCTGGAGTACAGCTCCTGTGACTATGGGTACCAACAGGGCC
TTTGCGTGCACTGCACACACACAGGGATTGAGTCCTGGATGTTATGACACCTATGCGG
CAGACATAGACTGCCAGTGGATTGATATTACAGATGTACAACCTGGAAACTACATTCT
AAAGGTCAGTGTAAA

(SEQ ID NO. 51)

FIGURE 12AV

CTATCAATGAAGGGGGAGATCACTGGGTAAGTTCGAATGCCCTCAGGCAAGGTGGCC
CAGCCTTCCATTACTGAATTCAAAGATGGCACTGTTACTGTACGTTACTCACCCAGTGA
AGCTGGCCTGCATGAAATGGACATTCGCTATGACAATATGCATATCCCAGGAAGCCCT
CTGCAGTTCTATGTTGATTATGTCAACTGTGGCCACATCACTGCTTATGGTCC

(SEQ ID NO. 52)

FIGURE 12AW

TTAGCACCTCGACCACGAAATGAGGAAGATGCAACAGACGTGGTGGGCCTGGCTCAG
GCTGTAAACGCTCGGTCCCCACCTTCAGTAAAACAGAACAGCTTGGATGAAGACCTTA
TTCGGAAGCTAGCTTATGTTGCTGCTGGGGACCTGGCACCCATAAAATGCTTTCATTGG
GGGCCTTGCTGCCCAGGAAGTCATGAAGGCCTGCTCTGGAAAAGTTTATGCCCATCATG
CAGTGGTTGTACTTTGATGCTCTTGAATGTCTCCAGAACGGACAAAGAGGCTCTGAC
AGAGGAGAGTGCTTCCCACGTCAGAACCGTTACGATGGGCAGGTAGCTGTATTGGTCA
GACTTCAGGAGAAGCTGAGAAGCAAA

(SEQ ID NO. 53)

FIGURE 12AX

TTAGCACCTCCAATGGCTGGGTACCAGCCAGCCGCAATGTCCGCTCCACAAATTTGGA
GTCTGTGAGGTACTGATTAACATTTTCTGCTGGCTGCTTGAAAAGGCCTTCAAATTCAT
CCCGGGCCCCACTGAAGAGTGTGTTGATGGCATTGGGAAAAGTTTTTTCAGGGTACAAAT
GGGGATGGATTTCTCTGGTGGATCCTGGCTAGACGTGATGGATTCTGTCAGGAAGGGG
ATTACCACCTGCACGTTGCCCTTT

(SEQ ID NO. 54)

FIGURE 12AY

TTAGCACCTCACACTCACATGCCCTTCTACATAGAGACTGGTTAAACAGCCCTCCCTCC
CTTGTCCTGACTTGACTTCCAGGCCCCCTCTGCTTTCCTCTCACAACCACACCAGGTCTG
ATGGAGTCCAGTGCCTGCAGTGACCCAACATAGACTGCACCTTCACCTACCTACTGGA
TGGTCTGCAGCCCAGACGGCTGCTCTTCTTCTCATGGAGTTTCTCTCCTGCCTGAGA
TATGCTATCTGGTCTGCCCCTGTGTAGCTCCCATGGGATCCCTTAAAATCGATCCTTTT
TTAA

(SEQ ID NO. 55)

FIGURE 12AZ

TTAGCACCTCGTGAGGAGACTGTTGTCCACAGGCCAGCTAGTGGTACCCCTACTGAGAA
GTTGGGTTTTGGTTTTGTTTCCCTTGAAGGGTCGCTGTTAGAGGATGGAAGTAACTTCT
AATTCTTGATCTGTTTGTGGTCTTGTTCAGTACTTTTGCCAGTTGTATACACTTGG
AGAGGGAATTTGTATGCCTGTAATCTTGTCTTGAGGTCAGAAATTCAAAACATTGGG
AGCTTTTGTGTAAAGGTTAAACTGTGAATCCATATAGCAAATGCAGATCCTTTTACA
GTGTAAACCACATTTCCCTGCCTCAGCCTAAAGCACTGGTCATTT (SEQ ID NO. 56)

FIGURE 12BA

ACCTGCATGCCTAAAGGAGTAGGCTTAGGGGTGGGGAGAGAGAAGGCATAGGCTTTT
CTAGTTATACAAAGCTGTGTAAGGCAAGGTTCCCTTTCTACTAAATGGTCAGCTGTCACT
ACATTTATACTTTTGTATGTCATAAACCTTTCTTTCAATTCCTCCCTGGGTAACCAGGA
CAATCGGAGGGCAGTGTGTTACTGGGATTAGAGGACTAGCAATACTGGGTAACCCGCC
TAAGCTGGAAGGTGACGTAATACGTTTCTTTAAAGATTCAGTCAGTCAAGCAGTTTAG
CAATATCAAAATGTCCTGGCTGTTTGGTCCAGTGTACACTGTT (SEQ ID NO. 57)

FIGURE 12BB

GCTATCTGCGAACTACAGAAAGGAAGACAGCTTGGCCCAGCGCGGTGAAGTTCAGA
ATTCACTAGGTAGTTGTTGTTGGTTGACTTGGAGGTAGCTGGGTAATCAACAGCTTTCA
CTTTAGATTCAATGTGAACCGCAGAGTTACTCATGACCAAGAGTCTGGCAAACCTCATT
AATGCTGTTTAACTTGTGTTGATATTTTTTCACCTTTTGAGCCCTTTTCCCAAAGAATT
CAATATCAGTTTAGTAGCAACAGTACAGTTGCCATTTAAATTGGTTTAGTTGCAGTATA
GCA (SEQ ID NO. 58)

FIGURE 12BC

GCTATCTGCGAACTACAGAAAGGAAGACAGCTTGGCCCAGCGCGGTGAAGTTCAGA
ATTCACTAGGTAGTTGTTGTTGGTTGACTTGGAGGTAGCTGGGTAATCAACAGCTTTCA
CTTTAGATTCAATGTGAACCGCAGAGTTACTCATGACCAAGAGTCTGGCAAACCTCATT
AATGCTGTTTAACTTGTGTTGATATTTTTTCACCTTTTGAGCCCTTTTCCCAAAGAATT

CAATATCAGTTTAGTAGCAACAGTACAGTTGCCATTTAAATTGGTTTAGTTGCAGTATA
GCA (SEQ ID NO. 59)

FIGURE 12BD

GCTATACTGCAACTAAACCAATTTAAATGGCAACTGTACTGTTGCTACTAAACTGATA
TTGAATTCTTTGGGAAAAGGGCTCAAAAAGGTGAAAAAATATCAAACAAGTATTAAAC
AGCATTAAATGAGTTTGCCAGACTCTTGGTCATGAGTAACTCTGCGGTTACACATTGAATC
TAAAGTGAAAGCTGTTGATTACCCAGCTACCTCCAAGTCAACCAACAACAACCTACCTA
GTGAATTCTGAACTTCACCGCGCTGGGCCAAGCTGTCTTCC

(SEQ ID NO. 60)

FIGURE 12BE

GCTATACTGCCCACCACATTGCCACACTCGGAATGACATTTCTATATTTTCACCTCCCC
AGATTTCCATTCTTTCATCGTAACTTCCAATGTGCTCAAAATATTTTTAGATAATAGAA
AAAAGGCCTCCTGCAAAGGTGGGGGTCTTAATTGGGTAGGTTTCATCTTTCCTTCTTTG
CTTCTCATGATCAGGAAGTGACTCCCAGCCAAAGGAAAGGCTCCAGTCAAAATTTCCA
CGGTTATGGTTGCTTCCGTACGGAGAAGGCTTGTGAATTCAAATGTGTTTAGATCTAT
GGATGCGATGTCTGGACTCACCACGGCA

(SEQ ID NO. 61)

FIGURE 12BF

GCTATACTGCTGAAGGAGATCATTTTGGTGGATGATGCTAGTGTAGACGACTACCTGC
ATGAAAAGCTGGAGGAATACATAAAACAGTTTTCTATTGTGAAAATAGTCAGGCAGC
AAGAAAGGAAAGGCCTGATCACCGCGCGGTTGCTAGGGGCAGCTGTAGCAACTGCCG
AGACGCTCACGTTCTTAGATGCTCACTGTGAGTGCTTCTATGGCTGGCTGGAACCTCTG
CTGGCCAGGATAGCTGAGAACTACACTGCCG

(SEQ ID NO. 62)

FIGURE 12BG

AGTTGCCAGGGGGCAGCTCACGGCGCAGCTCATCCTCTGTGATGTAATTCTTATCTCC
AGCCAGGATCTTGAAGGAAGCCATGACCTGATCTGCAGTATCAGTATCTGCCGTCTCT

CGGGACATAAAGTCGATGAAGGCCTGGAACGTCACTACCCCCAAGCGGTTGGGGTCT
ACAATGCTCATGATTCGGGCAAACCTCTGCCTCTCCCATGTTGTAACCCATGGAGATAA
GGCAGGCGCGGAAATCGTCTGTGTCCATCATGCCCCGTCTTCTTCCGGTCAAAGTGGTT
GAAAGA (SEQ ID NO. 63)

FIGURE 12BH

AAGCCGTGTCGCTGAACTGGGAGGACACACTGCTCACCCCTAGAAGGCTCTGGCTGACC
CTCCGCCCCGGTTAAACAGGGACTTTGTGGCCATGTGCTGGCGACACAGGTCTGGTAC
TCAAAAGTAGTGTCAACCATGGGCCCCCTCCGGCCCCAGCGCTGCCAGGCGTCCTTATC
CCGCTGTCTCGAATGATGGCGCATACCAAGGCCACTGAAAGCCACTAGCAGCCCAGCG
ACGCCTGCCAGGGCCACTAGAGTAAGCAGCACTGAGCGCATGGGAGATATGCCAT
(SEQ ID NO. 64)

FIGURE 12BI

AAGCCGTGCTGGACGTCCGTGTGTCCGGCTCTTGCTCACGCAGTCATGGCCTCCGGA
ACGCGCAAATCGGAAAGTCGGCTCCTGACTTCACGGCCACAGCGGTGGTGGATGGTGC
CTTCAAGGAAATCAAGCTTTCGGACTACAGAGGGAAGTACGTTGTCTCTTTTCTACC
CACTGGACTTCACTTTTGTGTTGCCCCACGGAGATCATCGCTTTTAGCGACCATGCTGAG
GACTTCCGAAAGCTAGGCTGCGAGGTGCTGGGAGTGTCTGTGGACTCTCAGTTACCC
ACCTGGCGTGGATCAATACCCACGGAAAGAGGGAGGCTT (SEQ ID NO. 65)

FIGURE 12BJ

AAGCCGTGTCGGAGGGCACCAAGGCTGTCACCAAGTACACCAGCTCCAAGTGAGTGC
TCAAGACTCAGCTCTTAACCCAAAGGCTCTTTTCAGAGCCACTCAAGACTTCAAAATT
GGAGCTTTAATGCTGACTTAGTGACTACCGGGAATAAAGTACTTCACTGTCAGGAT
TGTGTACAAACACTTATGGTTTAGTAAATCGAAAAGATAGACATTGCCCATCAGTTCT
GTCTGGTCCACTTAAATATGCTTTTTTCTTAGAAGTTCTAAGAACCCTGTCAATAACCT
ATCTAGGTCCAGTCCTTGAGTTCAAAGGCCAAATACCAATG (SEQ ID NO. 66)

FIGURE 12BK

CAACGCTCAGGATGTAAGCTGTTTCCAGCACCTGGTTCAAGCGAATGTAAGAAATAAG
AAGGTGTTGAAAGATGCCGTGAATAACATTACAGCAAAGGGGATCACAGATTACAAG
AAAGGCTTTAGCTTTGCCTTCGAACAGCTACTTAATTATAATGTTTCCAGAGCTAATTG
CAATAAGATTATCATGTTATTCACGGATGGAGGAGAAGAGAGAGCCCCAGGAGATATT
TGCCAAATACAATAAAGACAAAAAAGTCCGTGTGTTTACATTTCCGTGGTCAACAT
AATTATGACAGAGGACCTATTCAGTGGATGGCTTGTGAAATAAAGGTTACTATTATGA
GATTCCTCCATT (SEQ ID NO. 67)

FIGURE 12BL

TCAACGCTCATCACACCAAGAACTCAACTGGTTCTTCAAGTTTGTCTTATTTTCAGATTG
GCCAGTGACGTGAAGACTGGTAGAGTTCCAGTAATGACAAGTCCCAGTTCCAGGGCA
TCCAAATACACATTTGTCCATTGAACCTGCTTCGCTTTGTCACCAGCTAAAACCATTTGG
TCTTCCCAGAACATCTAGATATTCCTGAGTATTGATTCTTATTGCACCAATGGAGGGAA
TCTCATAATAGTAACCTTTATTTTCACAAGCCATCCACTGAATAGGTCTCTGTCATAAT
TATGTTGACCGACGGAAATGTAA

(SEQ ID NO. 68)

FIGURE 12BM

TAACGCTCAGGAGAAGAATAGGAATGCAGAGAACTCTGCCACAGCCCCACGCTCCC
GGGCAGCACCTCAGCCACCACCGCAACCACCCCTGCTGTAGATGAAAGCAAGCCT
TGAACCAAGTATCGCTTGCCTAAGACTCTTATACCTGACTCCTACCGGGTGATCTTGAG
ACCTTACCTCACCCCCAACAATCAGGGCCTGTACATCTTCCAAGGCAACAGTACTGTT
CGCTTTACCTGCAACCAGACCACGGATGTCATTATCATCCACAGCAAAAAGCTCAACT
ACACCTCAAAGGAAACCACAGGGTGG

(SEQ ID NO. 69)

FIGURE 12BN

CGAGTCAGACGGCTTCAGCATCGAGACCTGTAAGATCATGGTGGACATGCTGGATGAA
GATGGGAGTGGCAAGCTTGGCCTGAAGGAGTTCTACATCCTCTGGACGAAGATTGAGA
AATACCAAAAAATCTACCGGGAATCGATGTGGACAGGTCTGGAAGTATGAATTCCTA

CGAGATGCGGAAAAGCACTGGAAGAAGCAGGTTTCAAGCTGCCCTGTCAACTCCATCA
AGTCATCGTTGCCCGGTTTGCAGACGACGAGCTAATCATCGACTTTGACAATTTTG

(SEQ ID NO. 70)

FIGURE 12BO

CGAGTCAGACAACTGTTCAAGTGGGGTGGGGACCATCCACGGAGCAGCCGGCACCG
TATATGAAGACCTGAGGTACAACTCTCCCTAGAGTTCCCCAGCGGCTACCCCTTACAA
CGCACCCACAGTGAAGTTCCCTCACACCCTGCTACCACCCCAACGTGGACACCCAGGGC
AACATCTGCCTGGACATCCTCAAGGATAAGTGGTCTGCACTATATGATGTCAGGACTA
TCTTGCTCTCTATCCAGAGCCTGCTAGGAGAACCCAACATCGATAGCCTTTGAACACA
CACGCTGCGGAACTCTGGAAAA

(SEQ ID NO. 71)

FIGURE 12BP

TATGAGTCCGGAGCGACGGCTACGAGTGTGAAC TGTTCCAGCCCCGAGCGACACACCA
GAAGTTATGACTACATGGAAGGAGGGGATATAAGGGTGAGAAGACTGTTCTGTTCGCA
CCCAGTGGTACCTGAGGATTGACAAACGAGGCAAAAGTGAAAGGGACCCAGGAGATGA
AGAACAGCTACAACATCATGGAATCAGGACCGTGGCAGTTGGAATTGTGGCAATCA
AAGGGGTGGAAAGTGAATACTATCTTGCCATGAALCAAGGAAGGGAACTCTATGCAA
AGAAAGAATGCAATGAGGATTGCAACTTCAAAGAACTGATTCTGGAAAACCATTATA
ACACCTATG

(SEQ ID NO. 72)

FIGURE 12BQ

TATGAGTCCGAGGAGGAGCACAA TGCTGGGAGTGTGGAAAGCCAGGT TGTC CCCAGC
ACACACCGAGTGACCGATTCCAAGTTCCATCCACTCCATGCCAAGATGGATGTCATCA
AAAAAGGCCACGCCAGGGACAGCCAGCGCTACAAAGTTGACTATGAGTCTCAAAGCA
CAGACACCCAGAACTTCTCCTCCGAGTCTAAGCGGGAGACAGAATACGGTCCCTGCCG
CAGAGAAATGGAGGACACACTGAATCATCTGAAGTTCCTCAATGTGCTGAGTCCAGAG
TCTCACATCCAACTGTGACAAGAAGGGG

(SEQ ID NO. 73)

FIGURE 12BR

TCGCCCCGGGACTTCATGCGATTGAGAAGATTGTCTACCAAATATAGAACAGAAAAGAT
TTATCCCACAGCCACTGGAGAAAAAGAAAGAAAATGTTAAAAAGAACAGATATAAGGA
CATACTGCCATTGATCACAGCCGAGTTAAGTTGACTTTGAAGACTCCATCCCAAGAT
TCAGATTATATCAATGCAAAATTTTATTAAGGGTGTGTATGGGCCAAAAGCATATGTGG
CAACCCAAAGGGCCTTT

(SEQ ID NO. 74)

FIGURE 12BS

TGTGGAAGCCAGGTTGTCCCCAGCACACACCGAGTGACCGATTCCAAGTTCCATCCA
CTCCATGCCAAGATGGATGTCATCAAAAAAGGCCACGCCAGGGACAGCCAGCGCTAC
AAAGTTGACTATGAGTCTCAAAGCACAGACACCCAGAACTTCTCCTCCGAGTCTAAGC
GGGAGACAGAATACGGTCCCTGCCGCAGAGAAATGGAGGACACACTGAATCATCTGA
AGTTCCTCAATGTGCTGAGTCCAGAG

(SEQ ID NO. 75)

FIGURE 12BU

TGACCATCGAAGTGCAAAGGAAATGACTTGATTTCATGAAGTATCTCCAGAAGTAACG
CTTTGTTTTCTGCATCCTGAACTTTATTCCCAGTGAAGAGCTGAAAATCTGGACGCTCA
AAAAATGGAAGCACTTTGGAGAGAGCCCTTAACCTCTATCAGGTACAGGAAGTACAAG
TTCTCAGCCTTCGTGGGCCTTCTCCTTCAGTCAGAATCCCATCAAAGCGCTGCTGGAA
CTCTGTGACATTGTGACCCCATTTCTTTCCAGCCAAGTATCTTGTAAGATACCTTG
CACTCAAATGCACATTAATGCTTGCGTGCAGGCCAGATATAAGTCTGTAGAATCGCTC
TTTCTACACAGAGGCCTTCTAGCCAGTTGTAAA

(SEQ ID NO. 76)

FIGURE 12BV

CTGCTTGATGCTAAGCCCCGGCAGCCTGTGTTTCATCTACAGGATGCACAACATAAAAG
AAAAGATCTGATTCCCGCAGGTTCTCTTCTGACCTACACACACACACTAAAAATAAC
ATTTAAAAATATGTGCCAAATTAATTTGTTCCGGGTGCCACCTCCACCAGCTTACCAC
TACGGTAGAACTGTCAAATTCATCTCCCTGAATTTGTCTTAAAGGGGTGTCCATGCAC
AGGCCCAAGAGTCACTCCAATGAAAFAAATGTAATACTGAAGTATGCCATGATGTTT

GTGTTTCTTTCATCGTAAGCCTGTAAGCAGGAAAAATAGTAATAGATAGAATAGAG
ACTTACCAGTGGTCGATGGCCTGGTCAGTCTGTGCGGTGACTAGGACCAGG

(SEQ ID NO. 77)

FIGURE 12BW

ACCTGCATGCCGAGTGTGACGCCTTTGAGGAGAAGATCCAGGCTGCCGGAGGGATCG
AACTCTTTGTGCGAGGCCATTGGCCCCGATGGACACATTGCCTTCAATGAGCCAGGCTC
CAGCCTGGTGTCCAGGACCCGTGTGAAGACTCTGTTATGGACACCATCCTGGCCAAC
GCTAGGTTCTTTGATGGTGATCTTGCCAAGGTGCCACCATGGCCCTGACAGTGGGTG
TCGGCACTGTCATGGATGCTAAAGAGGTGATGATCCTCATCAGGGCGCTCACAAGGC
CTTTGCTCTGTACAAAGCCATCGATGGAGGCGTGAACCACATGTGGACGGTGTG

(SEQ ID NO. 78)

FIGURE 12BX

GCTATACTGCAATGTTAGGGGAATGAACGCGTTTTCCTACTGCACTGGGGACTTTTAG
ATAGGTTAATGAAAGGCCTTTTATTCTGTTACTGGACACGAAAACTTTGTCTAATTTCT
TATACTCTATTGTACGTTTACAGTCGCAGCACTAAAAATGGAAGACATCAAACATTTTT
AACAGAAAAAAAAAAAAAGATGTAAAACTAACTAAGGACTATTATTGATAATGTTTTG
CTACTCCTGTCAGACAATGGCTATAAACTGAATTAGGCAGTCTTAAAAAAAAAAAAAG
AAAAAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAG

(SEQ ID NO. 79)

FIGURE 12BY

AGCTAAGGTGCGGTACTCTGATACTTCAGAGTTTAAAAATCATCAGCCCTTGTAGATCT
ATTCTTAAATCTTATGAAAATGCTCAGATGTTTACACAGCTGTGAAACAGGGTCAGTT
CAGATCGCTGATGGCTTGAGAATGTGTTTCTTGTTGACATCAGGAACTGGAAATGTTT
ACTTCCCGTCATTTATGAGTCATCAAGTATCTCGGCTCTTTAAGAGCGCAAGATAAA
ACAAGCTTAAACCAGGTGATAAGAGCAGAGTCCACTTGAGTCTGAGCTACCCGAGA
ACTTGCTATCGAGGACATTTGGAATGGGAGTGTGCAGGCTTCCTTCAGTTACTGAATG
AGTCCATCTGCTAGTCACCTTGAC

(SEQ ID NO. 80)

FIGURE 12BZ

AGCTAAGGTCCAGGGGGCAAAGCGGTGACGTGTGCACATCGATATGAGAAACGGCAG
CACGTCAACACGAAGCAGGAGTCGCGGGATATCTTTGGAAGATGTTATGTCCTAAGTC
AGAATCTCAGAATTGAAGATGATATGGACGGAGGAGACTGGAGTTTCTGCGATGGCC
GGTTGAGAGGCCATGAAAAGTTTGGCTCCTGTCAGCAAGGAGTAGCGGCTACTTTTAC
TAAGGACTTTCATTACATTGTTTTTGGAGCCCCAGGGACTTACAACTGGAAAGGGATC
GTCGTGTAGAACAAAAGAATAACACTTTTTT

(SEQ ID NO. 81)

FIGURE 12CA

AAGCCGTGTCTGTGCTCAAGGAAGAAACCCACTGGACCAACTTCTGTCAGAAAGGAA
AACCTTGTTCAAAGTTTCAGGACCCCTGTTCTTTGCTTATTTGCACATGGTCACCTTGGT
CTGAGCTAGCCACCATTGTCACCCACAGCTGCAAAGAAAGCAGACCTTAGGAAACACT
GTCACGGCTGAGTGTGACTGCCTTGTTTCATCCCCTGGACTGGTACTGTGTTGCCTGCAG
TACCATTGGGATCCCATAGCAAGAGAGGGAGAGGGAGATGTTAGTTAGCCTTTGCTAC
GAACCAAGCTGTCCCAAGTCTCAACAGCTAAACAGGTATTCATTTACCATGATTCTAT
GGTTAGCTAAGCTCTTGAG

(SEQ ID NO. 82)

FIGURE 12CB

CTTTCTACCCTGGAGGATGTGCTTGAGGCACACTGCTCCTGTGCTCTCCACTTGAGGCA
TAAGCCCAGTCAGTTGTGCATAGATGATTAACCTCTGACCCCTAAAGATGGTAAGTTG
CTCTGGAGAAAGCATTTTAAACAGACAAACCAGGAGGCAAATCCCAACTTAGAGAGAT
GTTATCCACTGCACACTGTAGAGCAAACCTTGAGAGACCCAAGAGCCTTGGTCTGCATC
CTGTCCTTGCCCTGTGATAAACACTCGAGTACCCCTGATACCGGGCGATATTTTTGATT
AACTGGTCGAGGCTCCTTGTCCAATTCCAAAAGAGAACATCTGTGTTTC

(SEQ ID NO. 83)

FIGURE 12CC

TGGTAAAGGGCATCTGTAAATACACTCTATGAGGAAATTAAAACCTGAACATGGCAGT
CTGACATTGCAAAACAAAACAAAACAAAACCTGACCCTCCAATAGCAGCGAAAACAAC
GTGAAAGATACAAAGCAATGAGAATCTGGTTCTGAACGCCTGGGATCCTGGGAGTCAT
CGGTAGCAGCGCCATGAGAGGAGCCGTGGCCTGTCCCATGTGGTCCCACCTTCACCTC
TCCCTCACATCCCTCTTAAG

(SEQ ID NO. 84)

FIGURE 12CD

TGGTAAAGGGGGCAAGGGCAAAGGCACGGGAGACAGAGGCCACTGCATCTGTACCCA
CATCAGACATGTTTGTCCATTTCTCTCATTGGCCTTAGACCATTGGCAAGAGTAAAT
GCTCTTAGTCCCGTTATCTAGAAATTTCTTCCTTGGGGAGAACCACCTTATAGACAATA
TCAGCTCTCTACAAATAACACGAAAGGTCGTAACAC
AGCAAGTGACCAGAAAAGTGCCCGTCCTTGCGGCTCTGATCCACGTGGCTCTCCGTAGA
CAAATTGTTTTTCTTGAGGGATATCTGTTTTGCTTCTGAACCTTCTTACAAGTGTTTG
GGACTCTTCGGGTGGCGTT

(SEQ ID NO. 85)

FIGURE 12CE

TGGTAAAGGGTCAAGTGTTTCGATCAGAGTGGAGCTCCATTACCGAATGTAATCGTGGA
AGTCCAAGACAGAAAGCATATCTGCCCGTTTAGAACCAACAAGCTTGGAGAATACTAT
CTGCTTCTGCTGCCCGGGTCCTACGTGATCAATGTTACAGTCCCTGGACACGACTCCTA
CCTCACGAAGCTTACTATTCCAGGGAAATCCCAGCCCTTCAGTGCTCTAAAAAGGAT
TTTCACCTCCCGCTGCGATGGCAGCCGGATTCCATCTCCGTATCCAATCCTTCGTGCCG
ATGATTCCGCTGTACAAATTCATGCCAAGCCACTCGGCTGCCACAAAGCCTAGTCTGG
G

(SEQ ID NO. 86)

FIGURE 12CF

GAATTGGGCTTTCTGCGATCCACTCTTTGAAGCTATTGGCAAGATATTCAGCAACATCC
GCATCAGCACGCAGAAAGAGATATGAGGGACATTTCAAGGATGAAAGGTTTTTTTCCC
CCCTTACTATTTCCCTTGGTGCCAATTCGAAGTTGCTCTCGCAGCAGCAAATTTATGAAT

GGTTTGTCTTGATCAAGAACAAAGAATTCATTCCCACCATTCTCATATATACTACTTTC
TCTTCTT

(SEQ ID NO. 87)

FIGURE 12CG

GAATTCGGCTTTCTGCGATCCACTCTTTGAAGCTATTGGCAAGATATTCAGCAACATCC
GCATCAGCACGCAGAAAGAGATATGAGGGACATTTCAAGGATGAAAGGTTTTTTTCCC
CCCTTACTATTTCTTGGTGCCAATTCCAAGTTGCTCTCGCAGCAGCAAATTTATGAAT
GGTTTGTCTTGATCAAGAACAAAGAATTCATTCCACCATTCTCATATATCTACGTCTCT
TCTAG

(SEQ ID NO. 88)

FIGURE 12CH

ACGAGGGGAAACCTCCTCAGAGCCTGCAGCCAGCCACGCGCCAGCATGTCTGGGGGC
AAATACGTAGACTCCGAGGGACATCTCTACACTGTTCCCATCCGGGAACAGGGCAACA
TCTACAAGCCCAACAACAAGGCCATGGCAGACGAGGTGACTGAGAAGCAAGTGTATG
ACGCGCACACCAAGGAGATTGACCTGGTCAACCGCGACCCCAAGCATCTCAACGACG
ACGTGGTCAAGATTGACTTTGAAGATGTGATTGCAGAACCAGAAGGGACACACAGTTT
CGACGGCATCTGGAAGGCCAGCTTCACCACCTTCACTGTGACAAAATATTGGTTTTAC
CGCTTGTTGTCTACGATCTTCGGCATCCCAATGGCACTCATCTGGGGCATTACTTTGC
CATTCTCTCCTTCCTGCACATCTGGGCGGTTGTACCGTGCATCAAGAGCTTCCTGATTG
AGATTCAAGTGCATCAGCCGCTCTACTCCATCTACGTCCATACCTTCTGCGATCCACTC
TTTGAAGCTATTGGCAAGATATTCAGCAACATCCGCATCAGCACGCAGAAAGAGATAT
GAGGGACATTTCAAGGATGAAAGGTTTTTTTCCCCCTTACTATTTCTTGGTGCCAAT
TCCAAGTTGCTCTCGCAGCAGCAAATTTATGAATGGTTTGTCTTGATC

(SEQ ID NO. 89)

FIGURE 12CI

MECLYYFLGFLLLAARLPLDAAKRFHDVLGNERPSAYMREHNQLNGWSSDENDWNEKL
YPVWKRGD MRWKNSWKGGRVQAVLTSDSPALVGSNITFAVNLI
PRCQKEDANGNIVYEKNCRNEAGLSADPYVYNWTAWSESDGENGTGQSHHNVPDGGK

PPFHPGWRRWNFIYVFHTLGQYFQKLGRCSVRVSVNTANVTLPQLMEVTVYRRHGRA
YVPIAQVKDVYVVTQIPVFTMTQKNDRNSSIDETFLKDLPIFDVLIIDPSHFLNYSTIN
YKWSFGDNTGLFVSTNIITVNHTYVLNGTFSNLTVKAAAPGPCPPPPPPRPSKPTPSLGP
AGDNPLEI.SRIPDENCQINRYGHFQATITIVGILEVNIIQMIDVLMFVPWPRESSLIDFVVTC
QGSIPTEVCTIISDPTCEITQNTVCSPVDVDEMCLTVRRTFNGSGTYCVNLTGDDTSLAL
TSTLISVPDRDPASPLRMANSALISVGCLAIFVTVISLLVYKKHKEYNPIENSPGNVVRSGKI.
SVFLNRAKAVFFPGNQEKDPLLKNQEFKGV

(SEQ ID NO. 90)

FIGURE 12BT

1 CAGATGCCAG AAGAACACTG TTGCTCTTGG TGGACGGGCC CAGAGGAATT
CAGAGTTAAA
61 CCITGAGTGC CTGCGTCCGT GAGAATTCAG CATGGAATGT CTCTACTATT
TCCTGGGATT
121 TCTGCTCCTG GCTGCAAGAT TGCCACTTGA TGCCGCCAAA CGATTTTCATG
ATGTGCTGGG
181 CAATGAAAGA CCTTCTGCTT ACATGAGGGA GCACAATCAA TTAAATGGCT
GGTCTTCTGA
241 TGAAAATGAC TGGAATGAAA AACTCTACCC AGTGTGGAAG CGGGGAGACA
TGAGGTGGAA
301 AAACTCCTGG AAGGGAGGCC GTGTGCAGGC GGTCCTGACC AGTGACTCAC
CAGCCCTCGT
361 GGGCTCAAAT ATAACATTTG CGGTGAACCT GATATTCCCT AGATGCCAAA
AGGAAGATGC
421 CAATGGCAAC ATAGTCTATG AGAAGAACTG CAGAAATGAG GCTGGTTTAT
CTGCTGATCC
481 ATATGTTTAC AACTGGACAG CATGGTCAGA GGACAGTGAC GGGGAAAATG
GCACCGGCCA
541 AAGCCATCAT AACGTCTTCC CTGATGGGAA ACCTTTTCCT CACCACCCCG
GATGGAGAAG

601 ATGGAATTTC ATCTACGTCT TCCACACACT TGGTCAGTAT TTCCAGAAAT
TGGGACGATG

661 TTCAGTGAGA GTTCTGTGA ACACAGCCAA TGTGACACTT GGGCCTCAAC
TCATGGAAGT

721 GACTGTCTAC AGAAGACATG GACGGGCATA TGTCCCATC GCACAAGTGA
AAGATGTGTA

781 CGTGGTAACA GATCAGATTC CTGTGTTTGT GACTATGTTT CAGAAGAACG
ATCGAAATTC

841 ATCCGACGAA ACCTTCCTCA AAGATCTCCC CATTATGTTT GATGTCCTGA
TTCATGATCC

901 TAGCCACTTC CTCAATTATT CTACCATTAA CTACAAGTGG AGCTTCGGGG
ATAAATACTGG

961 CCTGTTTGTT TCCACCAATC ATACTGTGAA TCACACGTAT GTGCTCAATG
GAACCTTCAG

1021 CCTTAACCTC ACTGTGAAAG CTGCAGCACC AGGACCTTGT CCGCCACCGC
CACCACCACC

1081 CAGACCTTCA AAACCCACCC CTTCTTTAGG ACCTGCTGGT GACAACCCCC
TGGAGCTGAG

1141 TAGGATTCCCT GATGAAAACCT GCCAGATTAA CAGATATGGC CACTTTC AAG
CCACCATCAC

1201 AATTGTAGAG GGAATCTTAG AGGTTAACAT CATCCAGATG ACAGACGTCC
TGATGCCGGT

1261 GCCATGGCCT GAAAGCTCCC TAATAGACTT TGTCGTGACC TGCCAAGGGA
GCATTCCCAC

1321 GGAGGTCTGT ACCATCATTI CTGACCCAC CTGCGAGATC ACCCAGAACA
CAGTCTGCAG

1381 CCCTGTGGAT GTGGATGAGA TGTGTCTGCT GACTGTGAGA CGAACCTTCA
ATGGGTCTGG

1441 GACGTACTGT GTGAACCTCA CCCTGGGGGA TGACACAAGC CTGGCTCTCA
CGAGCACCT

1501 GATTTCTGTT CCTGACAGAG ACCCAGCCTC GCCTTTAAGG ATGGCAAACA
GTGCCCTGAT

1561 CTCCGTGGC TGCTTGGCCA TATTTGTCAC TGTGATCTCC CTCTTGGTGT
ACAAAAACA

1621 CAAGGAATAC AACCCAATAG AAAATAGTCC TGGGAATGTG GTCAGAAGCA
AAGGCCTGAG

1681 TGTCITTCTC AACCGTGCAA AAGCCGTGTT CTCCCGGGA AACCAAGAAA
AGGATCCGCT

1741 ACTCAAAAC CAAGAATTA AAGGAGTTTC TAAATTTCC ACCTTGTTTC
TGAAGCTCAC

1801 TTTTCAGTGC CATTGATGTG AGATGTGCTG GAGTGGCTAT TAACCTTTT
TTCCTAAAGA

1861 TTATTGTTAA ATAGATATTG TGGTTTGGGG AAGTTGAATT TTTATAGGT
TAAATGTCAT

1921 TTAGAGATG GGGAGAGGGA TTATACTGCA GGCAGCTCA GCCATGTTGT
GAAACTGATA

1981 AAAGCAACTT AGCAAGGCTT CTTTTCATTA TTTTATATGT TTCACCTATA
AAGTCTTAGG

2041 TAACTAGTAG GATAGAAACA CTGTGTCCC GAGTAAGGA GAGAAGCTAC
TATTGATTAG

2101 AGCCTAACCC AGGTAACTG CAAGAAGAGG CGGGATACTT TCAGCTTTCC
ATGTAACTGT

2161 ATGCATAAAG CCAATGTAGT CCAGTTTCTA AGATCATGTT CCAAGCTAAC
TGAATCCCAC

2221 TTCAATACAC ACTCATGAAC TCCTGATGGA ACAATAACAG GCCCAAGCCT
GTGGTATGAT

2281 GTGCACACTT GCTAGACTCA GAAAAATAC TACTCTCATA AATGGGTGGG
AGTATTTTGG

2341 TGACAACCTA CTTTGCTTGG CTGAGTGAAG GAATGATATT CATATATTCA
TTTATTCCAT

2401 GGACATTTAG TTAGTGCTTT TTATATACCA GGCATGATGC TGAGTGACAC
TCTTGTGTAT
2461 ATTTCCAAAT TTTTGTATAG TCGCTGCACA TATTTGAAAT CATAATTTAA
GACTTTCCAA
2521 AGATGAGGTC CCTGGTTTTT CATGGCAACT TGATCAGTAA GGATTTCACC
TCTGTTTGTA
2581 ACTAAAACCA TCTACTATAT GTTAGACATG ACATTCTTTT TCTCTCCTTC
CTGAAAAATA
2641 AAGTGTGGGA AGAGACAAAA AAAAAAAAAA //

(SEQ ID NO. 91)

FIGURE 12CJ

AAGGTGAAAGATGTGTATGTGATAACAGATCAGATCCCTGTATTTCGTGACCATGTCCC
AGAAGAATGACAGGAACTTGTCTGATGAGATCTTCCTCAGAGACCTCCCCATCGTCTT
CGATGTCCTCATTCATGATCCCAGCCACTTCCTCAACGACTCTGCCATTTCTACAAGT
GGAACCTTTGGGGACAACACTGGCCTGTTTGTCTCCAACAATCACACTTTGAATCACAC
TTATGTGCTCAATGGAACCTTCAACCTTAACCTCACCGTGCAAACCTGCAGTGCCCCGGG
CCATGCCCTCCCCCTTCGCCCTTCGACTCCGCCCTCCACCTTCGTA

(SEQ ID NO. 92)

FIGURE 12CK

AAGGTGAAAGATGTGTATGTGATAACAGATCAGATCCCTGTATTTCGTGACCATGTCCC
AGAAGAATGACAGGAACTTGTCTGATGAGATCTTCCTCAGAGACCTCCCCATCGTCTT
CGATGTCCTCATTCATGATCCCAGCCACTTCCTCAACGACTCTGCCATTTCTACAAGT
GGAACCTTTGGGGACAACACTGGCCTGTTTGTCTCCAACAATCACACTTTGAATCACAC
TTATGTGCTCAATGGAACCTTCAACCTTA

(SEQ ID NO. 93)

FIGURE 12CL

AAGGTGAAAGATGTGTATGTGATAACAGATCAGATCCCTGTATTTCGTGACCATGTCCC
AGAAGAATGACAGGAACTTGTCTGATGAGATCTTCCTCAGAGACCTCCCCATCGTCTT

CGATGTCCTCATTTCATGATCCCAGCCACTTCCTCAACGACTCTGCCATTTCTTACAAGT
GGAACTTTGGGGACAACACTGGCCTGTTTGTCTCCAACAATCACACTTTGAATCACAC
TTATGTGCTCAATGGAACCTTCAACCTTAACCTCACCGTGCAAACCTGCAGTGCCCCGGG
CCATGCCCTCCCCCTTCGCCTTCGACTCCGCCTCCACCTTCGTA (SEQ ID NO. 94)

FIGURE 12CM

TACGAAGGTGGAGGCGGAGTCGAAGGCGAAGGGGGAGGGCATGGCCCCGGGCACTGCA
GTTTGCACGGTGAGGTTAAGGTTGAAGGTTCCATTGAGCACATAAGTGTGATTCAAAG
TGTGATTGTTGGAGACAAACAGGCCAGTGTTGTCCCAAAGTCCACTTGTAGGAAAT
GGCAGAGTCGTTGAGGA

(SEQ ID NO. 95)

FIGURE 12CN

AAGGTGAAAGATGTGTATGTGATAACAGATCAGATCCCTGTATTCGTGACCATGTCCC
AGAAGAATGACAGGAACCTTGTCTGATGAGATCTTCCTCAGAGACCTCCCCATCGTCTT
CGATGTCCTCATTTCATGATCCCAGCCACTTCCTCAACGACTCTGCCATTTCTTACAAGT
GGAACTTTGGGGACAACACTGGCCTGTTTGTCTCCAACAATCACACTTTGAATCACAC
TTATGTGCTCAATGGAACCTTCAACCTTAACCTCACCGTGCAAACCTGCAGTGCCCCGGG
CCATGCCCTCCCCCTTCGCCTTCGACTCCGCCTCCACCTTCGTA

(SEQ ID NO. 96)

FIGURE 12CO

RRWRRSRRRRGRAWPGHCSLHGEVKVEGSIEHISVIQSVIVGDKQASVVPKVPLVGNR
VEEVAGIMNEDIEDDGEVSEEDLIRQVPVILLGHGHEYRDLICYHIHIFHL

(SEQ ID NO. 97)

FIGURE 12CP

KVKDVYVITDQIPVFVTMSQKNDRNLSDEIFLRDLPIVFDVLIIDPSHFLNDS
AISYKWNFG
DNTGLFVSNHTLNHTYVLNGTFNLNLTVQTAVPGPCPPSPSTPPPPS (SEQ ID NO. 98)

FIGURE 12CQ

YEGGGGVEGEGGGHGP GTAVCTVRLRLKVPLST*V*FKV*LLETNRPVI.SPKFHL*EMAES
LRKWLG S*MRTSKTMGRSLRKISSDKFLSFFWDMVTNTGI*SVITYTSFT (SEQ ID NO. 99)

FIGURE 12CR

MECI.YYFLGFLLLAARLPLDAAKRFHDVLGNERPSAYMREHNQLNGWSSDENDWNEKL
YPVWKRGMRWKNSWKGRVQAVLTSDSPALVGSNITFAVNLIFPRCQKEDANGNIVYE
KNCRNEAGLSADPYVYNWTAWSESDSGENG TGQSHHNVPD G K
PFPHPGWRRWNFIYVFHTL.GQYFQKLGRCSVRVSVNTANVTI.GPQLMEVTVYRRHGRA
YVPFAQVKDVYVVDQIPVFVTMFQKNDRNSSDETFLKDLPI MF DVLHDP SHFLNYSTIN
YKWSFGDNTGLFVSTNHTVNHTYVLNGTFSNLTVKAAAPGPCPPPPPPRPSKPTPSLGP
AGDNPLELSRIPDENCQINRYGHFQATITIVEGILEVNIIQMTDVLMPVPWPESLIDFVVT C
QGSIPTEVCTIISDPTCEITQNTVCSPVDVDEMCLLTVRRTFNGSGTYCVNLT LGDDTSLAL
TSTLISVPDRDPASPLRMAN S ALISVGCLAIFVTVISLLVYKKHKEYNPIENSPGNVVR SKGL
SVFLNRAKAVFFPGNQEKDPLLKNQEFKGVS (SEQ ID NO. 100)

FIGURE 12CS

1 CAGATGCCAG AAGAACACTG TTGCTCTTGG TGGACGGGCC CAGAGGAATT
CAGAGTTAAA
61 CCTTGAGTGC CTGCGTCCGT GAGAA TTCAG CATGGAATGT CTCTACTATT
TCCTGGGATT
121 TCTGCTCCTG GCTGCAAGAT TGCCACTTGA TGCCGCCAAA CGATTTCATG
ATGTGCTGGG
181 CAATGAAAGA CTTCTGCTT ACATGAGGGA GCACAATCAA TTAAATGGCT
GGTCTTCTGA
241 TGAAAATGAC TGGAATGAAA AACTCTACCC AGTG TGGAAG CGGGGAGACA
TGAGGTGGAA
301 AA ACTCCTGG AAGGGAGGCC GTGTGCAGGC GGTCTGACC AGTGACTCAC
CAGCCCTCGT
361 GGGCTCAAAT ATAACATTTG CGGTGAACCT GATATTCCT AGATGCCAAA
AGGAAGATGC

421 CAATGGCAAC ATAGTCTATG AGAAGAAGCTG CAGAAATGAG GCTGGTTTAT
CTGCTGATCC
481 ATATGTTTAC AAC'TGGACAG CATGGTCAGA GGACAGTGAC GGGGAAAATG
GCACCGGCCA
541 AAGCCATCAT AACGTCTTCC CTGATGGGAA ACCTTTTCCT CACCACCCCG
GATGGAGAAG
601 ATGGAATTTT ATCTACGTCT TCCACACACT TGGTCAGTAT TTCCAGAAAT
TGGGACGATG
661 TTCAGTGAGA GTTTCTGTGA ACACAGCCAA TGTGACACTT GGGCCTCAAC
TCATGGAAGT
721 GACTGTCTAC AGAAGACATG GACGGGCATA TGTCCCATC GCACAAGTGA
AAGATGTGTA
781 CGTGGAACA GATCAGATTC CTGTGTTTGT GACTATGTTT CAGAAGAACG
ATCGAAATTC
841 ATCCGACGAA ACCTTCCTCA AAGATCTCCC CATTATGTTT GATGTCCTGA
TTCATGATCC
901 TAGCCACTTC CTCAATTATT CTACCATTAA CTACAAGTGG AGCTTCGGGG
ATAATACTGG
961 CCTGTTTGTT TCCACCAATC ATACTGTGAA TCACACGTAT GTGCTCAATG
GAACCTTCAG
1021 CCTTAACCTC ACTGTGAAAG CTGCAGCACC AGGACCTTGT CCGCCACCGC
CACCACCACC
1081 CAGACCTTCA AAACCCACCC CTTCCTTAGG ACCTGCTGGT GACAACCCCC
TGGAGCTGAG
1141 TAGGATTCCT GATGAAAAC'T GCCAGATTAA CAGATATGGC CACTTTC AAG
CCACCATCAC
1201 AATTGTAGAG GGAATCTTAG AGGTTAACAT CATCCAGATG ACAGACGTCC
TGATGCCGGT
1261 GCCATGGCCT GAAAGCTCCC TAATAGACTT TGTCTGACC TGCCAAGGGA
GCATTCAC

1321 GGAGGTCTGT ACCATCATTT CTGACCCAC CTGCGAGATC ACCCAGAACA
CAGTCTGCAG
1381 CCCTGTGGAT GTGGATGAGA TGTGTCTGCT GACTGTGAGA CGAACCTTCA
ATGGGTCTGG
1441 GACGTA CTGT GTGAACCTCA CCCTGGGGGA TGACACAAGC CTGGCTCTCA
CGAGCACCTT
1501 GATTTCTGTT CCTGACAGAG ACCCAGCCTC GCCTTTAAGG ATGGCAAACA
GTGCCCTGAT
1561 CTCCGTTGGC TGCTTGGCCA TATTTGTCAC TGTGATCTCC CTCTTGGTGT
ACAAAAACA
1621 CAAGGAATAC AACCCAATAG AAAATAGTCC TGGGAATGTG GTCAGAAACA
AAGGCCTGAG
1681 TGTCTTCTC AACCGTGCA AAGCCGTGTT CTCCCGGGA AACCAGGAAA
AGGATCCGCT
1741 ACTCAAAAC CAAGAATTAA AAGGAGTTT TAAATTTTC ACCTTGTTT
TGAAGCTCAC
1801 TTTTCAGTGC CATTGATGTG AGATGTGCTG GAGTGGCTAT TAACCTTTT
TTCCTAAAGA
1861 TTATTGTAA ATAGATATTG TGGTTTGGGG AAGTTGAATT TTTTATAGGT
TAAATGTCAT
1921 TTTAGAGATG GGGAGAGGGA TTATACTGCA GGCAGCTTCA GCCATGTTGT
GAAACTGATA
1981 AAAGCAACTT AGCAAGGCTT CTTTCATTA TTTTATATGT TTCACCTATA
AAGTCTTAGG
2041 TAACTAGTAG GATAGAAACA CTGTGTCCCG AGAGTAAGGA GAGAAGCTAC
TATTGATTAG
2101 AGCCTAACC AGGTAACTG CAAGAAGAGG CGGGATACTT TCAGCTTTCC
ATGTAAGTGT
2161 ATGCATAAAG CCAATGTAGT CCAGTTTCTA AGATCATGTT CCAAGCTAAC
TGAATCCAC

2221 TTCAATACAC ACTCATGAAC TCCTGATGGA ACAATAACAG GCCCAAGCCT
GTGGTATGAT
2281 GTGCACACTT GCTAGACTCA GAAAAAATAC TACTCTCATA AATGGGTGGG
AGTATTTTGG
2341 TGACAACCTA CTTTGCTTGG CTGAGTGAAG GAATGATATT CATATATTCA
TTTATTCCAT
2401 GGACATTTAG TTAGTGCTTT TTATATACCA GGCATGATGC TGAGTGACAC
TCTTGTGTAT
2461 ATTTCCAAAT TTTTGTATAG TCGCTGCACA TATTTGAAAT CATATATTAA
GACTTTCCAA
2521 AGATGAGGTC CCTGGTTTTT CATGGCAACT TGATCAGTAA GGATTTCCAC
TCTGTTTGTA
2581 ACTAAAACCA TCTACTATAT GTTAGACATG ACATTCTTTT TCTCTCCTTC
CTGAAAAATA
2641 AAGTGTGGGA AGAGACAAAA AAAAAAAAAA // (SEQ ID NO. 101)

FIGURE 12FN

MECLYYFLGFLLLAARLPLDAAKRFHDVLGNERPSAYMREIHNLNGWSSDENDWNEKL
YPVWKRGMRWKNSWKGRVQAVLTSDSPALVGSNITFAVNI.IFPRCQKEDANGNIVYE
KNCRNEAGLSADPYVYNWTAWSESDGNGTGQSHHNVFPDGKPFPHHPGWRRWNFIY
VFHTLGQYFQKLGRCSVRVSVNTANVTLGQPLMEVTVYRRHGRAYVPQAQVKDVYVVT
DQIPVFVTMFQKNDRNSSDETFLKDLPIMFQVLIHDP SHFLNYSTINYKWSFGDNTGLFVS
TNHTVNHTYVLNGTFSNLTVKAAAPGPCPPPPPPRPSKPTPSLGPAGDNPLELSRIPDEN
CQINRYGHFQATITIVEGLEVNIIQMTDVLMPVPWPSSLIDFVVTTCQGSIPTEVCTIISDPT
CEITQNTVCSPVDVDEMCLLTVRRTFNGSGTYCVNI.TLGDDTSLALTSTLISVPDRDPASP
LRMANSALISVGCLAIFVTVISLLVYKKHKEYNPIENSPGNVVRSGKLSVFLNRAKAVFFPG
NQEKPPLLKNQEFKGV* (SEQ ID NO. 102)

FIGURE 12CT

CTGACCAGGAACCCACTCTTCTGTGCATGTATGTGAGCTGTGCAGAAGTATGTGGCTG
GGAAGTGTGTCTCTAAGGATTATTGTAAAATGTATATCGTGGCTTAGGGAGTGTGG
TTAAATAGCATTTTATAGAGAAGAAAAAAAAAAAAAAAAAACTCGAGAGTACTTCTAG
AGCGGCCGCGGCCATCGATTTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAA
TTTCGCCTATAGTGAGT (SEQ ID NO. 103)

FIGURE 12CU

AGGACAAGCCAAGGACACTCTAAGTCTTTGGCCTTCCCTCTGACCAGGAACCCACTCT
TCTGTGCATGTATGTGAGCTGTGCAGAAGTATGTGGCTGGGAAGTGTGTCTCTAAG
GATTATTGTAAAATGTATATCGTGGCTTAGGGAGTGTGGTTAAATAGCATTTTAGAGA
AGACATGGGAAGACTTAGTGTCTTCTCCCATCTGTATTGTGGTTTTTAACTGTTCGTG
GGGTGGACACGCTGTGTCTGAAGGGGAGGTGGGGGTCACTGCTACTTAAGGTCCTAGG
TTAACTGGGGGAGATACCACAGATGCTCAGCTTTCACATAACATGGGCATGAACCAG
CTAATCACACTGAA (SEQ ID NO. 104)

FIGURE 12CV

GGATCCTTCTCCTGGTCTCCTCGGAAGAACGGGGCTTTCGCGTGACTGAGGAGAACAC
TCAGGCCCTTGCCCTTGACCGTGTCTCTGGGGCAGTTTCCTATTGGCTTGACGCCTTG
TGTTTTTTGTACAGCAAGATGGTAACCATGGTGACAAGCACAGCCAGGCAGCCGATGG
AGATCAGGACACCATTCACTGCTCTCAGAGGGAGTCTGGGTCTTTGCCAGGGATAGAG
ATCAGGGTGCTGGTGAGGGCCAGGCTTCGATCATCTCCCAGAGTGAAATTCACACAGT
AGGTGCCAGACCCATTGAAGGCTCTTCTCACAGACAGCAGCACAGCCATCCACAGCC
ACAGGGCTGCAGACCCGGTTCTGGGCGATCTGGCAGGTGGGGTCGGAGATGATCGTA
CAGGCTTCCATGGGGGTGGCCCCCTTTCAGGTCACAGTGAAGTCCATCAGGGAGTTGG
CAGGCTGCGGTGTGGGCATGGGGACATCTGCTATCTGCATGATGCTGACTTCCAGGATCC
(SEQ ID NO. 105)

FIGURE 12CW

TAGCAGATGTCCCATGCCACACCGCAGCCTGCCAACTCCCTGATGGACTTCACTGT
GACCTGCAAAGGGGCCACCCCATGGAAGCCTGTACGATCATCTCCGACCCCACTGC
CAGATCGCCCAGAACCGGGTCTGCAGCCCTGTGGCTGTGGATGGGCTGTGCTGCTGTC

TGTGAGAAGAGCCTTCAATGGGTCCTGGCACCTACTGTGTGAATTTCACTCTGGGAGAT
GATCGAAGCCTGGCCCTCACCAGCACCTGATCTCTATCCCTGGCAAAGACCCAGACT
CCCTCTGAGAGCAGTGAAT (SEQ ID NO. 106)

FIGURE 12CX

GGATCCTTCTCCTGGTCTCCTCGGAAGAACGGGGCTTTCGCGTGACTGAGGAGAACAC
TCAGGCCCTTGCCCTTGACCGTGTTCCTGGGGCAGTTTCCTATTTGGCTTGTACGCCCTTG
TGTTTTTTTGTACAGCAAGATGGTAACCATGGTGACAAGCACAGCCAGGCAGCCGATGG
AGATCAGGACACCATTCACTGCTCTCAGAGGGAGTCTGGGTCTTTGCCAGGGATAGAG
ATCAGGGTGCTGGTGAGGGCCAGGCTTCGATCATCTCCAGAGTGAAATTCACACAGTA
(SEQ ID NO. 107)

FIGURE 12CY

TTTTTTTTTTTTTTTTTAGACTGCCTTTTAAATGAGTAGAATATGTACACACACGCACC
ATACACAAAGCCCGGGCCATTATAATTTTGTGAGGAGCTCAGGCATGCTCAGTGAGT
TGGAAGGCAGATGAAGCATG
CCTTCAGGTGGTGATTAGCTGGGTTCATGCCCATGTTATCGTGGAAAGCTGAGGCATC
TGTGGTATCTCCCCAGTTAACCTAGGACCTTAAGTAGCAGTGACCCACCTCCCTTCAG
ACACAGCG (SEQ ID NO. 108)

FIGURE 12CZ

GGATCCTGGAAGTCAGCATCATGCAGATAGCAGATGTCCCATGCCACACCCGAGCC
TGCCAACTCCCTGATGGACTTCACTGTGACCTGCAAAGGGGCCACCCCATGGAAGCC
TGTACGATCATCTCCGACCCACCTGCCAGATCGCCCAGAACCGGGTCTGCAGCCCTG
TGGCTGTGGATGGGCTGTGCTGCTGTCTGTGAGAAGAGCCTTCAATGGGTCTGGCACC
TACTGTGTGAATTTCACTCTGGGAGATGATCGAAGCCT (SEQ ID NO. 109)

FIGURE 12DA

TTTTTTTTTTTTTTTTTTCTTCTCTAAAATGCTATTTAACCACACTCCCTAAGCCACGA
TATACATTTTACAATAATCCTTAGAGAAACAACAGTTCCCAGCCACATACTTCTGCACA
GCTCACATACATGCACAGAAGAGTGGGTTCCCTGGTCAGAGGGAAGGCCAAAGACTTA
GAGTGTCTTGGCTTGTCTGGAGCAATGGATCCTTCTCCTGGTCTCCTCGGAAGAACG
GGCTTT (SEQ ID NO. 110)

FIGURE 12DB

AAACTGCAGTGCCCGGGCCATGCCCTCCCCCTTCGCCTTCGACTCCGCCTCCACCTTCA
ACTCCGCCCCCACCTCCGCCCTCACCTCTGCCACATTTATCAACACCTAGCCCCCTCTTT
AATGCCTACTGGTTACAAATCCATGGAGCTGAGTGACATTTCCAATGAAAACCTGCCGA
ATAAACAGATATGGCTACTTCAGAGCCACCATCACAATTGTAGAGGGGATCCTGGACG
CAGCATCATGCAGATAGCAGATGTCCCATGCCACACCCGAGCCGTCCAACCTCCTGAT
GGACTTCACTGTGACCTCAAGGGCACCCATGGAAGCTGTCAGA (SEQ ID NO. 111)

FIGURE 12DC

CCTCAACGACTCTGCCATTTCTTACAAAGTGGAACCTTTGGGGACAACACTGGCCTGTTT
GTCTCCAACAATCACACTTTGAATCACACTTATGTGCTCAATGGAACCTTCAACCTTAA
CCTCACCGTGCAAACCTGCAGTGCCCGGGCCATGCCCTCCCCCTTCGCCTTCGACTCCGC
CTCCACCTTCAACTCCGCCCTCACCTCCGCCCTCACCTCTG (SEQ ID NO. 112)

FIGURE 12DD

CCTCAACGACTCTGCCATTTCTTACAAAGTGGAACCTTTGGGGACAACACTGGCCTGTTT
GTCTCCAACAATCACACTTTGAATCACACTTATGTGCTCAATGGAACCTTCAACCTTAA
CCTCACCGTGCAAACCTGCAGTGCCCGGGCCATGCCCTCCCCCTTCGCCTTCGACTCCGC
CTCCACCTTCAACTCCGCCCTCACCTCCGCCCTCACCTCTGCCACATTATCAACACCT
AGCCCCCTCTTTAATGCCTACTGGTTACAAATCCATGGAGCTGAGTGACATTTCCAATG
AAAACCTGCCGAATAAACAGATATGGCTACTTCAGAGCCACCATCACAATTGTAGAGG
GGATCCTGGAAGTCAGCATCATGCAGATAGCAGATGTCCCATGCCACACCCGAGCC
TGCCAACTCCCTGATGGACTTCACTGTGACCTGCAAAGGGGCCACCCCCATGGAAGCC
TGTACGATCATCTCCGACCCACCTGCCAGATCGCCAGAACCGGGTCTGCAGCCCTG

TTCTGCGATCCCTGCTCCGTCGAGAGACCTTCATCGGCTCCACCACTGCGGATTCACTCCGAGATATCAACCT

(SEQ ID NO. 113)

FIGURE 12DE

GGATCCCCCTCTACAATTGTGATGGTGGCTCTGAAGTAGCCATATCTGTTTATTCGGCAG
TTTTCATTGGAAATGTCACCTCAGCTCCATGGATTTGTAACCACTAGGCATTTAAAGAGG
GGCTAGGTGTTGATAATGTGGGCAGAGGTGAGGGCGGAGGTGAGGGCGGAGTTGAAG
GTGGAGGGCGGAGTCGAAGGCGAAGGGGGAGGGCATGGCCCGGGCACTGCAGTTTGCA
CGGTGAGGTTAAGGTTGAAGGTTCCATTGAGCACATAAGTGTGATTCAAAGTGTGATT
GTTGGAGACAAACAGGCCAGTGTTGTCCCAAAGTCCACTTGTAGGAATGGCAGAGTC
GTTGAGG

(SEQ ID NO. 114)

FIGURE 12DF

CCTCAACGACTCTGCCATTTCTACAAGTGGAACCTTGGGGACAACACTGGCCTGTTT
GTCTCCAACAATCACACTTTGAATCACACTTATGTGCTCAATGGAACCTTCAACCTTAA
CCTCACCGTGCAAACCTGCAGTGCCCGGGCCATGCCCTCCCCCTTCGCCTTCGACTCCGC
CTCCACCTTCAACTCCGCCCTCACCTCCGCCCTCACCTCTGCCCACATTATCAACACCT
AGCCCCCTCTTAATGCCTACTGGTTACAAATCCATGGAGCTGAGTGACATTTCCAATG
AAAACCTGCCGAATAAACAGATATGGCTACTTCAGAGCCACCATCACAAATTGTAGAGG
GGATCCTGGAAGTCAGCATCATGCAGATAGCAGATGTCCCCATGCCACACCCGAGCC
TGCCAACTCCCTGATGGACTTCACTGTGACCTGCAAAGGGGCCACCCCCATGGAAGCC
TGTACGA

(SEQ ID NO. 115)

FIGURE 12DG

GAAGGTGGAGGCGGAGTCGAAGGCGAAGGGGGAGGGCATGGCCCGGGCACTGCAGTT
TGCACGGTGAGGTTAAGGTTGAAGGTTCCATTGAGCACATAAGTGTGATTCAAAGTGT
GATTGTTGGAGACAAACAGGCCAGTGTTGTCCCAAAGTTCCACTTGTAGGAAATGGC
AGAGTCGTTGAGGAAGTGGCTGGGATCATGAATGAGGACATCGAAGACGA

(SEQ ID NO. 116)

FIGURE 12DH

GAATTTCGCACGAGGGGAGTCAGAGTCAAGCCCTGACTGGTTGCAGGCGCTCGGAGTC
AGCATGGAAAAGTCTCTGCGGGGTCTGGGATTTCTGCTGCTGGCTGCAGGACTGCCTC
TCCAGGCTGCCAAGCGATTTTCGTGATGTGCTGGGCCATGAACAGTATCCCGATCACAT
GAGAGAGCACAAACCAATTACGTGGCTGGTCTTCGGATGAAAATGAATGGGTTCCAATA
TCACTTTTGTGGTGAA (SEQ ID NO. 117)

FIGURE 12DI

GAATTTCGGCACGAGGAAGGAGGCCGTGTGCAGGCAGTCCTGACCAGTGACTCACCGG
CTCTGGTGGGTTCCAATATCACTTTTGTGGTGAACCTGGTGTTCCTCCAGATGCCAGAAG
GAAGATGCTAATGGCAATATCGTCTATGAGAAGAACTGCAGGAATGATTTGGGACTG
ACATCTGACCTGCATGTCTACAACCTGGACTGCAGGGGCAGATGATGGTGA CTGGGAAG
ATGGCACCT (SEQ ID NO. 118)

FIGURE 12DJ

GAAGGTGGAGGCGGAGTCGAAGGCGAAGGGGGAGGGCATGGCCCCGGGCACTGCAGTT
TGCACGGTGAGGTTAAGGTTGAAGGTTCCATTGAGCACATAAGTGTGATTCAAAGTGT
GATTGTTGGAGACAAACAGGCCAGTGTGTCCCCAAAGTTCCACTTGTAGGAAATGGC
AGAGTCGTTGAGGAAGTGGCTGGGATCATGAATGAGGACATCGAAGACGATGGGGAG
GTCTCTGAGGAAGATCTCATCAGACAAGTT (SEQ ID NO. 119)

FIGURE 12DK

GAATTTCGGCACGAGGTCAAGCCCTGACTGGTTGCAGGCGCTCGGAGTCAGCATGGAA
AGTCTCTGCGGGGTCTGGGATTTCTGCTGCTGGCTGCAGGACTGCCTCTCCAGGCTGC
CAAGCGATTTTCGTGATGTGCTGGGCCATGAACAGTATCCCGATCACATGAGAGAGCAC
AACCAATTACGTGGCTGGTCTTCGGATGAAAATGAATGGATGAACACCTTGTATCCA
(SEQ ID NO. 120)

FIGURE 12DL

AAGGGGGAGGGCATGGCCCGGGCACTGCAGTTTGCACGGTGAGGTTAAGGTTGAAGG
TTCCATTGAGCACATAAGTGTGATTCAAAGTGTGATTGTTGGAGACAAACAGGCCAGT
GTTGTCCCCAAAGTTCCACTTGTAGGAAATGGCAGAGTCGTTGAGGAAGTGGCTGGGA
TCATGAATGAGGACATCGAAGACGATGGGGAGGTCTCTGAGGAAGATCTCATCAGAC
AAGTTCCTGTCATTCTTCTGGGACATGGTCACGAATACAGGGATCTGATCTGTTAT

(SEQ ID NO. 121)

FIGURE 12DM

GAATTCGGCACGAGCCGACACTGTGACTCCTGGTGGATGGGACTGGGGAGTCAGAGT
CAAGCCCTGACTGGTTGCAGGCGCTCGGAGTCAGCATGGAAAGTCTCTGCGGGGTCTT
GGGATTTCTGCTGCTGGCTGCAGGACTGCCTCTCCAGGCTGCCAAGCGATTTCTGTGAT
GTGCTGGGCCATGAACAGTATCCCGATCACATGAGAGAGCACAAACCAATTA

(SEQ ID NO. 122)

FIGURE 12DN

AAGGTGAAAGATGTGTATGTGATAACAGATCAGATCCCTGTATTCTGTACCATGTCCC
AGAAGAATGACAGGAACCTGTCTGATGAGATCTTCCTCAGAGACCTCCCCATCGTCTT
CGATGTCCTCATTTCATGATCCCAGCCACTTCCTCAACGACTCTGCCATTTCTACAAGT
GGAACTTTGGGGACAACACTGGCCTGTTTGTCTCCAACAATCACACTTTGAATCACAC
TTATGTGCTCAATGGAACCTTCAACCTTAACCTACCGTGCAAACCTGCAGTGCCCCGGG
CCATGCCCTCCCCCTTCGCCTTCGACTCCGCCTCCACCTTCGTA

(SEQ ID NO. 123)

FIGURE 12DO

TACCATCGGAGAAAGAAGACCAAGCAAGGCTCAGGCAGCCACCGCTGCTTCGCACT
GAGCCTCCTGACTCAGACTCAGAGTCCAGCACAGACGAAGAGGAATTTGGAGAATTG
GAAATCGCTCTCGTTTTGTCAAGGGAGACTATCCCGATGCTGCAAGATCTGCTGTCCCT
CTGGCCTTTGTCATCCTCGCGCTTGGCTTGTGGCCTCTGTGGGCTTGGTGTGGAGCAAA
TGGCTCTCAAGGAGGACTGAGTCTCAAGGAAATT

(SEQ ID NO. 124)

FIGURE 12DP

AGCTAAGGTCAGGAGGTGTCTGAAGAATTGGCTGATGCATGGCAGGGATGTTGTTGAC
CTGCTTTTAGAACAATACTTCCATTTAATTATAGCATATCTTATGTGTGTATTAAAGCA
GAGCCGATCTGGTGGGGCTCATTAAAGTAAATGTACTTACTGCAAAAGGTTCAACTGGT
GAUCCCCAGTTTTCCCCAGAAGCAATATGATAGGACAGAGGCGACTCCTGCAAGTTGTC
TCAGACTTCACACATACATTGTGACATTCTCTGAGCATGTGC'ACTGTACATGATATGAC
ACTATCAA (SEQ ID NO. 125)

FIGURE 12DQ

AGCTAAGGTCCACTACCTTGTGAAGATGTATAAACACCTGAAATGTAGAAGCGATCCG
TATGTCAAGATCGAGGGGAAGGACGCTGACGACTGGCTGTGTGTGGACTTTGGGAGTA
TGGTGATCCATTTGATGCTTCCAGAAACCAGAGAAACCTATGAATTAGAGAAACTATG
GACTCTACGTTCTTTTGATGACCTTAGCTAAGCCGAATCAGCACACTGGCGGCGTTACT
AGTGGATCGAGCTCGTACAGCTGATGCATAGCTTGAGTA'CTATAGGTTACTAATAGC
TGGCTATCATGTCAAGCGTTC (SEQ ID NO. 126)

FIGURE 12DR

GCTGAGCTGCAGAGAGTAGCACATCCTTGCTAAT'CAATAA'CTACCAGTTTTTATTGGT
GAAACATGAATCCAGATGGTATGGTTGCTCTCCTGGACTACCGTGAAGATGGTGTGAC
TCCATT'CATGATTTTCTTTAAGGATGGCTTAGAGATGGAGAAATGTTAACAAATTGGA
TCTATCACCTGTCACCATAAT'GGCTGCTGCTTACCATCCATACAACACCAGGACTTAG
GACAAATGGGACTGATGTCATCTTGAGCTTTTATTTTGACCTTAGCT (SEQ ID NO. 127)

FIGURE 12FO

AGCTAAGGTCAGAGCCAATAGTATCATGAGAACTGAAGAAGTAATAAAGCAACTTCT
CCAGAAATTTAAGATTGAGAATAGCCCTCGGGATTTGCTCTTTACATTATTTTGGGA
CAGGAGAGCAGAGAAAGCTAAAGAAGACCGATGTCCACTGCTGCAGAGGTTACTACA
AGGACCATCCAAAAGCAATGCTCGGATCTCTCATGGATAAAGATGCAGAAGAATCAC
GAGAGATGTGGCTCGTACATTATTTCACTTTCTTCTGATCATACTCAAGATAGATGAGA
GAGAAT (SEQ ID NO. 128)

FIGURE 12DS

TTGACTTCTGAGTCTAACACAGACACTGCAAGGGTTAATTTTCCAAGAGGTGGTTGT
GTTGACGATAAATTCATTAAGAATTTTAAAAATTTAGTTAGATTTACCAAAGTCACTG
GAGACAAATTCAGAAGGCATATATACCTGCCAGTTTGTGGACTACATTAATAGGGAG
GCTTTTATGTTTGATGTAATTCTTACAGTTCTAAGAATTAAGTTCCATTGCATGAGACC
TTAGCT (SEQ ID NO. 129)

FIGURE 12DT

AAGGTGAATCCCCGACGGCTCTGGGCCCAGGAGAAGCGTCGCCGTGGCAAATTGGC
ACTGCAGGAGAAGCCCTCCACAGGTA CTGGAAAACTGGTCTCTGAGGCCAAGGCC
AGCTCCGAGACATTCAGGACTTCTGGATCAGCCTCCAGGGACACTGTGCAGTGAGAAG
ATGGCCATGAGTCCTGCCAGTGAG (SEQ ID NO. 130)

FIGURE 12DU

AATTTTTTTTTTCGACGGCCCAACGGGGGCTTGGTGGATGGAAATATGGTTTTGTGAGT
TATTGCACTACCTGGAATATCTATGCCTCTTATTTGCGTGTA CTGTGCTGCTGATCGT
TTGGTGCTGTGTGAGTGAACCTATGGCTTAGAAAAACGACTTTGTCTTAACTGAGTG
GGTGTTCAGGG (SEQ ID NO. 131)

FIGURE 12DV

CACCTGATTAAAGGAAAAGCATTCTGACGTAAGAAGCTGAAAGGCGGCCCTTGCGTG
CTTTGAACTTTCTTATACAGCACAGTCATCTGAAGCTTCCTGTGTGACCAAGACAAGA
ACGCGTGCAACAAGACTGAGAAACAGCAAGAAACAACCCGGCATTCTACTTTCTCAAC
ACTATCATACTTTAAACCTTTTAC (SEQ ID NO. 132)

FIGURE 12DW

CTAGCTTACGCTAGTCCCCCATGCATAAAGACTGATCGCTTTTCCTTAGAAAGGTGAG
AGGGTTAGGACAAGGCCGTGTGGTAACAACACCCGCAGCTCGAAAAACCAATGGCTT
GTTAACGTGTCAGTGAGGCACTGTACGGACGTCCATAGTCCACATCTTCAAATTCCCG
CAGAAGGCTTCCTATTCTTAAACTCTA

(SEQ ID NO. 133)

FIGURE 12DX

CTACATTTCTGTATCCATTCTCTGTTGAAGGCTCTGGTTCTTCCAGCTTCTGGCTATT
ATAAATAAGGCTGCTATAAACACAGTGGAGGCATGTGTCTTGTATATTTTGGAGCA
TCTTTTGGGTATATGCCCAGAAGTGCTATAGCTGGTTCCTCAGGTAGTACTATGTCGAA
TTTTCTGAGGAACTGCCAGACTGATTTCCAGAGTGGTTGTACCAGCTTGCAATCCCACC
AGCAATAGAGGAGTGTTCCTCTTCTCTATATTCTTGCCAACATCTGCTGTCACCTGAG
TGTTT

(SEQ ID NO. 134)

FIGURE 12DY

TGGTAAAGGGGAATGATGTCGAGGCCATCCTGGGCTGTAGAGCCAGGCCCTGGCTTG
GGGAGTGGGCATTGTTAACTTGTTGCTGACTTTGTGTTGACCCCTGCATCAGCAACTAT
TTCCTTAAATCCAGGATACAACTTGTTAAGTGTGACAGCTTTCCTTTACACACCATTTT
TGTGGGTGTATATATATATTTGACTTGGGGAGAATTATTTTTTACAAAAATACAAAAT
AGCTTTTAA

(SEQ ID NO. 135)

FIGURE 12DZ

AGCTAAGGTCCGGACTCTATGGCATGACCCCAAAAACATTGGCTGGAAAGATTACACT
GCCTACAGGTGGCACCTGATTCACAGGCCTAAGACAGGCTACATGAGAGTCTTAGTGC
ATGAAGGAAAGCAAGTCATGGCTGACTCAGGACCAATTTATGACCAAACCTACGCTG
GTGGACGGCTGGGCTGTTTGTCTTCTCCAAGAGATGGTCTATTCTCGGACCTCAAGTAT
GAGTGCAGAGATGCTAGAGAGCAGGCTCAGTCTCAGCA

(SEQ ID NO. 136)

FIGURE 12EA

TGACCTACGTGTAGTTGGTGTGCTTGTGTGCGAAGATGAGGGCCTCCTGGATGAGCTG
GTGCTGCTGCTCCAGCAGGTCCAGGCTGGGCTTGTAGTCCACGAGTCTGCGCTCGTAC
TGCTTCAGGTGGCTCAGCTGGTCTTCCAGAGTCCCGTTCATCTCAATGGAGATGCGCCC
GATCTCCTCCATCTTAGTCTGGATCCACGGCCCCACCATATTGGCTTGGCTGGCGAACT
GTCGGCGAAGGCTGCATTGGATTGCT

(SEQ ID NO. 137)

FIGURE 12EB

AATTTTTTTTTTCGACGGCCCAACGGGGGCTTGGTGGATGGAAATATGGTTTTGTGAGT
TATTGCACTACCTGGAATATCTATGCCCTCTTATTTGCGTGTACTGTTGCTGCTGATCGT
TTGGTGCTGTGTGAGTGAACCTATGGCTTAGAAAAACGACTTTGTCTTAACTGAGTG
GGTGTTCAAGG

(SEQ ID NO. 138)

FIGURE 12EC

CACCTGATTTAAAGGAAAAGCATTCTGACGTAAGAAGCTGAAAGGCGGCCCTTGCGTG
CTTTGAACTTTCTTATACAGCACAGTCATCTGAAGCTTCCTGTGTGACCAAGACAAGA
ACGCGTGCACAAGACTGAGAAACAGCAAGAAACAACCCGGCATTCTACTTTCTCAAC
ACTATCATACTTTAAACCTTTCAC

(SEQ ID NO. 139)

FIGURE 12ED

CTAGCTTACGCTAGTCCCCCATGCATAAAGACTGATCGCTTTTCCTTAGAAAGGTGAG
AGGGTTAGGACAAGGCCGTGTGGTAACAACACCCGCAGCTCGAAAAACCAATGGCTT
GTAAACGTGTCAGTGAGGCACTGTACGGACGTCCATAGTCCACATCTTCAAATTCCCG
CAGAAGGCTTCCTATTCTTAACTCTA

(SEQ ID NO. 140)

FIGURE 12EE

CTACATTTCTGTATCCATTCTCTGTTGAAGGCTCTGGTTCTTTCCAGCTTCTGGCTATT
ATAAATAAGGCTGCTATAAACACAGTGGAGGCATGTGTCCTTGTTATATTTTGGAGCA
TCTTTTGGGTATATGCCCAGAAGTGCTATAGCTGGTTCCCTCAGGTAGTACTATGTGCAA

TTTTCTGAGGAAGTCCAGACTGATTTCCAGAGTGGTTGTACCAGCTTGCAATCCCACC
AGCAATAGAGGAGTGTTCCCTCTTCTCTATATTCTTGCCAACATCTGCTGTCACCTGAG
TGTTT (SEQ ID NO. 141)

FIGURE 12EF

TGGTAAAGGGGGAATGATGTCGAGGCCATCCTGGGCTGTAGAGCCAGGCCCTGGCTTG
GGGAGTGGGCATTGTAACTTGTTGCTGACTTTGTGTTGACCCCTGCATCAGCAACTAT
TTCCTTAAATCCAGGATACAACCTGTAAAGTGTGACAGCTTTCCTTTACACACCATTTT
TGTGGGTGTATATATATATTTGACTTGGGGAGAATTATTTTTTACAAAAATACAAAAT
AGCTTTTAA (SEQ ID NO. 142)

FIGURE 12EG

AGCTAAGGTCCGACTCTATGGCATGACCCCAAAAACATTGGCTGGAAAGATTACACT
GCCTACAGGTGGCACCTGATTCACAGGCCTAAGACAGGCTACATGAGAGTCTTAGTGC
ATGAAGGAAAGCAAGTCATGGCTGACTCAGGACCAATTTATGACCAAACCTACGCTG
GTGGACGGCTGGGCTGTTTGTCTTCTCCAAGAGATGGTCTATTCTCGGACCTCAAGTAT
GAGTGCAGAGATGCTAGAGAGCAGGCTCAGTCTCAGCA (SEQ ID NO. 143)

FIGURE 12EH

TGACCTACGTGTAGTTGGTGTGCTTGTTGTGCGAAGATGAGGGCCCTCCTGGATGAGCTG
GTGCTGCTGCTCCAGCAGGTCCAGGCTGGGCTTGTAGTCCACGAGTCTGCGCTCGTAC
TGCTTCAGGTGGCTCAGCTGGTCTTCCAGAGTCCCGTTCATCTCAATGGAGATGCGCCC
GATCTCCTCCATCTTAGTCTGGATCCACGGCCCCACCATATTGGCTTGGCTGGCGAACT
GTCGGCGAAGGCTGCATTGGATTGCT (SEQ ID NO. 144)

FIGURE 12EI

TGACCATCGATAAGTTTAATAACTACAGACTTTTCCCAAGACTACAAAAGCTTCTTGA
AAGTGACTACTTTAGATATTACAAGGTGAACCTGAAGAAGCCTTGTCTTTCTGGAAT

GACATCAACCAGTGTGGAAGAAGAGACTGTGCCGTCAAACCCTGCCATTCTGATGAAG
TTCTTGATGGAATTAAGTCTGCCGAGCTACAAGTATTCTG
AGGAAGCCCAACCGCATTGAAGAAATGTGAGCAAGCTGAGCG (SEQ ID NO. 145)

FIGURE 12EJ

AACTCTGTGAACCGTGCCTTTCTCTGTGGAGGTGGAGGTGTCGGTTGAAGACAAGCGA
GGTCCTCCAAGGGGCTGTGTCTTATGTTGCCATCTCCCCTTGTAAGCTTGGCTGCCCACC
CTCCAGACTGTGCGCCATGGCTCCAAGGCTGTGACCCGCCACTGGAGTCATGCACTTC
CAGCGGCAGAAGCTGATGCTATAACTGAGTATAATCCTCCAAACCTGCCATCAACCCG
AGA (SEQ ID NO. 146)

FIGURE 12EK

ACTTCTCCAGAGAATTTAAGATTGAGAATAGCCCTCGGGATTTGCTCTTTACATTATT
TTTGGGACAGGAGAGCAGAGAAAGCTAAAGAAAGACCGATGTCCCACTGCTGCAGAGG
TTACTACAAGGACCATCCAAAAGCAATGCTCGGATCTTCTCATGGATAAAGATGCAG
AAGAAATCAGCAGAGATGTGGCTCCGTACATTAATTTCACTTTTCTTTCTTGATCCAT
CCTTCAAGATTAGATGAAGAAAGAGAAATGGAGATTGAGAGAATATGCAATCATACCGA
(SEQ ID NO. 147)

FIGURE 12EL

AGGGTTACTTCAGGCTAAGGCAATAGAAATCCATTTAAGATGGTGTGCTAAAGGCTT
GATGGATGTTCAATCGTCTGTCTAAAGGAGAATGAAGTCATCAACAGGATGTCAGGGGA
AAGTGAGATCATCGCAGAAAAGTATCAACTTAGCACAAACACACAGGCATAGCTCCTG
CAAGAGGTGAATGCTGTCCCCAAATACCTGAGGAACTATCCCTTTGGGCAAGAAAATA
GACAAGTCCATGAAGTCTGGGTGA
(SEQ ID NO. 148)

FIGURE 12EM

GACCAGGTACACTTGAGCAAAGCACCCAGTATTTAATTCCTTACAGAAAGGAGAGGA
AAGGTCTGCAGTTGGAAGTATGCTAACACCGCAAATGACTGTCAATTTGATCTC

AGAAGTTCAGGATTGATTGCTATGTTTTAGCTCTAATTGTGAGAAACAGTAGTCATTTT
AGTCTTAAATTTTGCCTCAGGAAATTCAGGGAGACTGAGCCTTCCTTCCCCACCTTC
GTAAAGCCGAATTCAGCACACGGCGGCCGTTACTAGTGGATCCGAGCTCG

(SEQ ID NO. 149)

FIGURE 12EN

TACAAGGTGGGATGGCAGGAACGAAGGCTTCTGTAAATCCAGTTTTGGCTCTCTCTC
TGGTCTTTCTTTCTTTCTGTTCTGTTTGAAGGGTTTCTGGTCTTTCAGGAGGTATTTT
TTTAATTTTCATGTTTTCTCTCTGTGGTACCTGCCCCCTGTTTGACGACAGGAGCTGATG
GAGGTGGCGGTTTCTTGGGTCTATTCCCTTCCTTGTCAAAGTCCGATGGAAGTAACTTC
ACGAAGTTGTCAGGAAACACGCCTCGTCTGCCATTGAGTTCTCCTTCCCACCAGCCTA
CGCGATGCAGTCTTATTGATGAGAGTCACTATATCTCCTTA

(SEQ ID NO. 150)

FIGURE 12EO

TCACCCATGACTTCTATGGACTTGTCTATTTTCTTGCCCAAAGGGATAGTTCCCTCAGGT
ATTTGGGGACAGCATTACCTCTTGCAGGAGCTATGCCTGTGTGTTTGTGCTAAGTTGA
TACTTTCTGCGATGATCTCACTTTCCCTGACATCCTGTTGATGACTTCATTCTCCTTA
GACAGACGATGAACATCCATCAGGCCTTTATGCACACCATCTTAAAATGGATTTCTAT
TGCCTTAGCCTGAAGTCC

(SEQ ID NO. 151)

FIGURE 12EP

CCCATAGAGATAGGTTTGCTCCAGAACCTGCAGCATTTCACATCACAGGGAACAAGG
TGGACATTCTGCCAAAACAGTTGTTTAAGTGCGTGAAGTTGAGGACTTTGAACCTGGG
GCAGAACTGTATCGCCTCCCTGCCTGAGAAAATCAGTCAGCTCACCCAGCTCACTCAG
CTGGAGCTGAAGGGCAACTGCCTAGACCGCCTGCCAGCCCAGCTGGCAGTGTGATGC
TCAAGAAGA

(SEQ ID NO. 152)

FIGURE 12EQ

CAATAATCCAGGTAAAAATAGAGTAAAAATAGTCTGCTAGCAGCAAGTTCCTACCATACT
TTCAACAACACTCACGAGATACGGAATGATTACAGCATTAAAGAAATATTTAGAAAATGA
CAGGTAGGTGTGGTGGACAGGTGGCTCACATTCAAGACTCAAGTCTACTTAAAAAAGA

AAATCTCACTAGCACTAGATTCTAGCTCCTTTGTTTCCCCCTTCTTTTGGTTTCAAAG
GCGTTTCTACAACCCATAAGAGG

(SEQ ID NO. 153)

FIGURE 12ER

GCCAAGCTATTATGACACTATAGATACTCAACGTATCGATCAACGTTGGTACCGAGCT
CGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTCGGCTTGGATTGGTCAGAGCA
GTGTGCAATATGATCCAATAAGTCTCCTCCCTTGGCCCCCTCCCCAAAATGTTTGCAGT
GTTATTTTTGTGGGTTTTTTTTTAACACCCCTGACACCTGTTGTGGACATTGTCAACCTTT
GTAAGAAAACCCAAATAAAAATTGAAAAATAAAATAAAAAGAAACCCATGAACATTC
GCACCACTTGTGGCTTCTGACTATCTTCCACAGAGGGAAGTTTAAACCCAAACTTCC
AAAGGTTTGAACCTACCTCAAGACACTTTTCGCAGTGGAGTCGTAGACCAATCCCA

(SEQ ID NO. 154)

FIGURE 12ES

TAAATAAATTAAAAAATTAATAAACCTAAAAACGTCCACCAAACCTAAAACCATTAA
ACAACCAACAAACCCACTAACAATTAAACCTAAACCTCCATAAATAGGTGAAGGCTTT
AATGCTAACCCAAGACAACCAACCAAAAAATAATGAACTTAAAACAAAAATA

(SEQ ID NO. 155)

FIGURE 12ET

GGTAAAGGGGACCTGGAGAACGCCTTCCTGAACCTGGTCCAGTGCATCCAGAACAAG
CCCCGTGACTTCGCTGACCGGCTGTACGACTCCATGAAGGGCAAGGGGACTCGAGACA
AGGTCTGATTAGAAATCATGGTCTCTCGCAGTGAAGTGGACATGCTGAAAATCAGATCT
GAATTCAAGAGGAATATGGCAAGTCCTGTACTACTACAT

(SEQ ID NO. 156)

FIGURE 12EU

AGAGCAGCAGGCCAGCTGTACTTGGTTTGGCAAGAAAAAGAAAGCAGTACAAAGATAA
ATATTTGGCAAAGCACAACGCAGTGTTTGATCAATTAGATCTTGTACATATGAAGAA
GTAGTCAAACCTGCCAGCATTCAAAAGGAAACATTAGTCTTATTAGGTGCACATGGTG
TTGGAAGAAGACACATAAAAATACCCATCACAAAGCAC

(SEQ ID NO. 157)

FIGURE 12EV

TCCGGTCATAGTAGTAAGGGAAATCTCCCAGGTAAAGATGAATACTGCGGTAGGACGAA
CAATCCTCCAGGATGTTTGTTCATATTAAACTGTTACGTGATATGTGCTTGAATATTC
TGTCTTGAATAATCTCTAGTGTAGTTAATACAATCTTCTCAACTGAAGAAAAATAAGC
CTCCACAAGAAGCTGTGTCTGCTGTCTAAGTGCTAGGATTTTATCCTGATGAATAGACC
TGATTGTAGAAGGAATCTGTAATAGCAATCTCTCATCGCCTATGACCGAAAGCCGAAT
TCTGCAGATATCCATCACACTGGCCGGCCGCTCGAGCATCGATCTAGAGGG

(SEQ ID NO. 158)

FIGURE 12EW

CTGCTTGATGACAAAGGGTGTAGTCTTCATCTTTTCTGGATTATTTTGGAAAGTGACAG
GTGGAAATTCATCGTCACGTTTATGTGGTCTGTAAAGCCAACGATCTCAAATTCGG
CGGCTCAAGAGGAGCGTTTGCAGGCACGATGTAGTCTGAGCAGCGGCACACGGTCAA
GTCCCTCTGTGCACTATGACGATGGCGACGACGTAGCTCTCCATGCCCTCCAACCAC
TTATCTGTCACGTCACATGATGACTTCGTGGTATCTGAACAGTTCTTAACCTTCGTCAG
ATTTTCGTCTTT

(SEQ ID NO. 159)

FIGURE 12EX

AAATCGTTGCTTCAGAAAGACTCAATAACACTTACTTGTGCCTGGCTGTGCTGACAGT
ACATTCTGTGTCATTTTCTTCATGGCGGAACAGTCCACAGAGCTCACCAACAAGTA
CTCCAAAAGTGAAGAGTTTAAGCTTCGAGATGCAACCAGATGAGCTTCTAGAAAA
GCCCATGTCTCCCATGCAGTACGCACGGTCTGGACTAGGGACAGCAGAGATGAATGGC
AAACTCATAGCTGCAGGTGGTTATAACAGAGAGGAATGTCTTCGAACAGTTGAATGCT
ATGATCCACATACAGATCACTGGTCCTTCCTTGCTCCCATGAGAACATCAAGCAG

(SEQ ID NO. 160)

FIGURE 12EY

CTTTCCGAAGAGCACACCCTCCTCTCAATGAGCTTGTGAGGTCTCTTTCTTCTCTTCCT
TCCAACGTGGTGTAGCTCCAGGCGAGCGACGTGAGAGTGCCACCTGAGACAGACAC
CTTGGTCTCAGTTAGAAGGAAGATGCAGGTCTAAGAGGAATCCCCGAGGTCTGTCTG
AGCTGTGATCAAGAAATATTCCGCAATGTGCCTTTTCTGAGATCGTGTTAGCTCCAAAG

CTTTTTCCTATCGCAGAGTGTTTCAGTTTGTGTTTGTGTTTGTGTTTGTGTTTGTGTTTGTGTTTTC
CCTTGGCGGATTTCCTCGTGCT (SEQ ID NO. 161)

FIGURE 12EZ

CCTATTGAACGGTCTTGCAATGACGAGCATTCAGATGCTTAAGGAAAGCATTGCTGCT
ACAAATATTTCTATTTTAGAAAGGGTTTTATGGACCAATGCCCCAGTTGTCAGTCAA
AGCCGTTGGTGTTTTATTGTTTAAATGTCACCTATAAAACGGGCATTATTTATGTTT
TTTTCCCTTTGTTTCATATTCTTTGCATTCCTGATTATTGTATGTATCGTGTAAGGAA
GTCTGTA (SEQ ID NO. 162)

FIGURE 12FA

CCTATTGAACGGTCTTGCAATGACGAGCATTCAGATGCTTAAGGAAAGCATTGCTGCT
ACAAATATTTCTATTTTAGAAAGGGTTTTATGGACCAATGCCCCAGTTGTCAGTCAA
AGCCGTTGGTGTTTTATTGTTTAAATGTCACCTATAAAACGGGCATTATTTATGTTT
TTTTCCCTTTGTTTCATATTCTTTGCATTCCTGATTATTGTATGTATCGTGTAAGGAA
GTCTGTA (SEQ ID NO. 163)

FIGURE 12FB

CCTGGGTCCGTCTCCAACCCCTCAGCCCCAAACCCTCCGACTTCACTTCTTGAAGTG
ATCGGAAAGGGCAGTTTTGGAAAGGTTCTTCTGGCTAGGCACAAGGCAGAAGAAGTA
TTCTATGCAGTCAAAGTTTTACAGAAGAAGCCATCCTGAAGAAGAAAGGAAGGAAGC
ATATTATGTCAGAGCGGAATGTTCTGTTGAAGAATGTGAAGCACCCCTTTCCTGGTGGG
CCTTCACTTCTCATTCCAGACCGCTGACAAGCTCT (SEQ ID NO. 164)

FIGURE 12FC

GATGCTGAACACAAAAAGAAAGAAAAAGGAAGAGGAGGAGCAAGAGAAGCTGAA
GGGAGGGAGCCTTGGCGAAAATCAGATCAAAGATGAGAAGATTAAAAAGGACAAAG
AGCCCCAAAGAGAGTCAAGAGCTTCTTGGATAGAAAGAAAGGATTTACAGAGTGAGG
CGCAGAATGGAGATTCAATGACCCACAACTTAAAC

(SEQ ID NO. 165)

FIGURE 12FD

AAAGCCAATTGGTAGAGAAATTGAAGACACAAATGCTGGATCAGGAAGAGCTTCTGG
CATCAACCAGAAGGGATCAAGATAATATGCAAGCTGAACTGAAATCGCCTCCAAGCAG
AAAATGATGCTTCTAAAGAAGAGTAAAGAGTTTACAGGCCTTAGAGGACTGCTGTTA
ATTATGATCAGAGTTCAGGAGTTAAGAC

(SEQ ID NO. 166)

FIGURE 12FE

CTGCTTGATGTCCTGTGTAGCGAATGTCACAGCGTACAACATTGTTAGTGTAGTCTGAT
TCAGGCACCAGGTAGCTGGGGTTTACACTGACCTTTAGAATGTAGTTTCCAGGTTGTA
CATCTGTAATATCAATCCACTGGCAGTCTATGTCTGCCGCATAGGTGTCATAACATCCA
GGACTCAATCCCTGTGTGTGTGCAGTGCACGCAAAGGCCCTGTGGTACCCATAGTCAC
AGGACGTGTCCTCCAGACAGAAGCTTGCTTTGTGGCCTTCAGCCACTCTCCTCTGTGTG
TTGGCATCAACGAGAAGCCGAATTCTCGAGATATCCATCACACT (SEQ ID NO. 167)

FIGURE 12FF

CTGCTTGATGTCCTGTGTAGCGAATGTCACAGCGTACAACATTGTTAGTGTAGTCTGAT
TCAGGCACCAGGTAGCTGGGGTTTACACTGACCTTTAGAATGTAGTTTCCAGGTTGTA
CATCTGTAATATCAATCCACTGGCAGTCTATGTCTGCCGCATAGGTGTCATAACATCCA
GGACTCAATCCCTGTGTGTGTGCAGTGCACGCAAAGGCCCTGTGGTACCCATAGTCAC
AGGACGTGTCCTCCAGACAGAAGCTTGCTTTGTGGCCTTCAGCCACTCTCCTCTGTGTG
TTGGCATCAACGAGAAGCCGAATTCTCGAGATATCCATCACACT (SEQ ID NO. 168)

FIGURE 12FG

GATCTGACACTACAGCATGAGCGTTAGATTTTATAAAATTAATTTTCTTCTAAATGCTG
GAAACTCTAAGGGTTTATTCAGAAAAAACTGGCCAAATTTCAAATGGCTTAGAAGC
AGGGTTAATTAAGTATTGAATGAGCCACTGTGATATCCTGATGACACCCAGTCACAAT
GACAGTTTTGAAGCATACAACCAAAACAATTGAGATCTCAAAACTATTTTACATCACT
TATGGTAATGTTATGTAAAAATGAAAATGCTTTCTGTGGAAGTTACATTCTTTACCAGG
TCTTTAACATAAAATTAACACGACGTCGAGTAAGCCTTGTTCGGAAGACAAACTAGTT
TGTGAGTTCAGTCAGATCCCAGCT (SEQ ID NO. 169)

FIGURE 12FH

AGTTGCCAGGACCACCACCATAGTTGCCAGGTCATCATAAACAAATCCAACATCAAT
CTTAAATTCCCCCATCAGACAATCTGCCCTCAAAGAATGGGAATTATAAACCCGGATA
CTGATGATCTCATCCATGAGCTCAGAGGGTGTGATGTGCACATTGTAGAAAAATAACT
CGTCAAAAAACGGATTGTTCCCTCTCTTGATTCTCGTGCGATGCGTCTGACCACAGATG
TGAACCTTCACCACGGGCCTTATGTTGTTGCCGCATAACTGACGGCCCTCGATCACTCT
GACACGGATCTGGAAATCTGTGGCTTGTGGACAGCATCCTT (SEQ ID NO. 170)

FIGURE 12FI

AAGCCGTGTCCCAAAGAATGGATAGAGACGCGATCAGATGCGACAGTGCTGTGGAGA
AAGCCCAGGAACCTGCACAATTGCCCTGGTCCAATGGCTCGTGGATCAGGTTGGGCCA
CTTCTCTGAAGCTTCAAAGGCAGTGGGTAGCACTTCCCCCTTGGCCCAGCACCGTATAA
ATCTCATTCATATTCATGACAGTGGAGGATGGGCGGATTGTGCCCAGGCGGTACGGAA
TGCCCTCATCCAGGGTCATGCCCCAGAAGGCACTGTGGTTCCCAGCCTGCCACCCGTA
GTTGCCTCGGTTGATGGCTTTAATCATGTCTGGTCACTAGACACGGCTTAAGCGAATCT
CGAGATATCCATCACACTGGCGGCGTTCGAGAT (SEQ ID NO. 171)

FIGURE 12FJ

AAGCCGTGTCTGATGATGGAGGTAGTGGTGGGGGAGGAGGGACTGAGGGTCTGAGG
TGGTGGCCCCTGGAACCTGATCCCACATAGTTACCCACTGCTAGTTCTGACCCCGTGG
CAACGTGCCAGAGGCCATGACTGGCAGTATGGCAATGTCCCCATCCCCCTTCTTCTTA
ATTTTAATGGTCCCTTGTCTTCTCCAGTTTCGTGAATCTTTTTTCCAGGGTAGACTGTCTT
TGAATGGCTTCTTCCCTTTCTTTGACCATTTTCTTAAACGTGTGAACTTGGGTATTGCA
TCTTTGTAGATTTCCGGACAACATCAGTTCCTTATTCCTCTGCATAAGTTGCTTTCAGTT

(SEQ ID NO. 172)

FIGURE 12FK

CGAGTCAGACACATGAAAGCAAAACGCGGGCAGATAAAACGATCGCCTTACCTTCTA
GCAAAAATCTGAAGCTTGTGTGAGAAACAAAGACTCAGAAAGGTTTGTCTTCAGATGA
AGAAGACTCTGAGGATTGTCTTCTCTCAAAGTTCAAGTAAGCAAAAAGTGCATCA

CTTTCATCCAGCCAGCCCCCAACATCAGTCTCCCTTTTGGTGATGAAGATGAAGAGG
ACAGTCTTTTGGGAGTGCAGCAGCTAAGAAGCAGACTTCATCTCTACAACCTCAGAG
TCAAGAGAAAGCAAAGCCTTCCGAGCAGCCCTCAAAGAAGACATCTGCCTTGTGTTTC
AGA

(SEQ ID NO. 173)

FIGURE 12FL

CGAGTCAGACTTAATTTAAAAACGAAACAAAACAAAAATAACATAGTTTAGAAATCA
AGGAGAAAGGACAGATAGTCTAAGAAAAAGACAACACAAAAGAGGGGCAGGGCGG
CCAGCTTGCATCAGGGATCTTGGCTGGAGACCTGCTTTGAATAGGTTTCTTGCAGGTAT
TTCTTAAATGCTGTGGGGTTTTTCCAGAGTTCCGCAGCGTGTGTGTTCAAAGGGCTATC
GATGTTGGGTTCTCCTAGCAGGCTCTGGATAGAGAGCAAGATAGTCCTGACATCATAT
AGTGCAGACCACTTATCCTTGAGGATGTCCGGCAGATGTTGCCCTGGGTGTCACGTTGG
GGTGGTAGCAGGGTGTGAGGAACTTCACTG

(SEQ ID NO. 174)

FIGURE 12FM

CGAGTCAGACACTCCTGGCTCCTGGATTCTTTAGATGCCTCCATCAGACTGGGTACTTT
AGATGCCTCCATCAGACTACTTCGTCATTGTATTTCTCAGTTCGCTCAGGGCAAGCGGC
AGTCTCTGGGCTGCTGTGGCAGGTGCCACCACTGCATTTAAAAGTTAAAATTTCTTCA
AATATTCCCATCAAGGCCTTGAGCCTCTGAGATTGGTTTACTATTTGCCCAGTTATTT
AAAGCTCTCTGCATTCTTCCTGATTTAATATTGCTATGGCCAGGACAATGTGTAGAAG
TAAAAAGGATATCATATTTACAGGTGTAACGC

(SEQ ID NO. 175)

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DD-PCR primer and PCR size (nt)	cDNA from Cell line	Mouse homology (%nt)	Human homology (%nt)	Northern (P-MT) (screen 1)	Northern-cloned DNA (P-MT) (screen 2)
P17-8 c10 (1100)	151-1 LM1	mouse nicotinic acetylcholine receptor alpha (54.3%)		no	151-1LM1 up, 151-1 LMA down
P19-8 c2 (500)	151-2 PA		lymphocyte IgE receptor (52.6%)	no	151-2LMA down,down
P21-8 c3 (450)	151-2 PA	histon H2b (94.2%)		151-1LM1 down,down	151-1LM1 down,down
P21-9 c6 (500)	151-1 PB	Rattus norvegicus thiol- specific antioxidant mRNA(94.4%)		151-1LM1 down,down 151-2LMA up,up	151-1LM1 down,down 151-2LMA up,up
P21-17 c9 (1000)	148-1 LMD	Mus musculus putative protein tyrosin phosphatase mRNA(98.3%)		148-1LMD up,up 151-1LM1 up,up	148-1LMD up,up 151-1LM1 up,up
P22-5 c3 (500)	148-1 LMD	Rat dihydropyridine-sensitive L-type calcium channel alpha-2 subunit gene (92.5%)		148-1LMD up,up	148-1LMD up,up
P22-8 c4 (500)	148-1 LMD	same as P22-5 c3		148-1LMD up 151-1LM1 up	148-1LMD up,up
P22-9 c3 (500)	148-1 LMD	Rat kidney Zn-peptidase aminopeptidase N mRNA (90.5%)		148-1LMD up,up,up	148-1LMD up,up,up
P24-8 c3 (550)	151-1 PB		ubiquitin carrier protein (E2-EPF) mRNA (33.3%)	151-1LM1 down 151-2LMA up 151-2LMB up	151-2LMA up
P24-10 c5 (1400)	151-1 LM1	Rattus norvegicus caspase II 80 kDa subunit mRNA (83%)		151-1LM1 up,up	151-1LM1 up,up
P25-1 c3 (400)	148-1 PA	M. musculus keratinocyte growth factor Fgf-7 (99.4%)		148-1LMD down 151-1LM1 down,down 151-2LMB up,up 151-2LMA up	148-1LMD down 151-1LM1 down 151-2LMB up 151-2LMA up
P25-9 c8 (1300)	151-1 PB	M. musculus mRNA for insulin-like growth factor binding protein-3(98.1%)		148-1LMD up 151-1LM1 down,down,down 151-2LMA up,up,up	148-1LMD up 151-1LM1 down,down,down 151-2LMA up,up,up
P2-27 (c18-3)	148-1 PA	rattus norvegicus glycogen mRNA (93.4%)			148-1LMD down P23(+/-)2 down

FIGURE 13A

Clone #	cDNA from Cell Lines	DO Primer	PCR Size (nt)	Mouse Homology	Human Homology	Northern blot #	Regulation Type	Sequencing Primer	Sequencing Length
Cl 381 Cl 481 (same frag & orientation)	151-2 LMB	P3		Tyrosine Kinase? Vip2	Caveolin (70%)	N123 148-1 up 151-1 up 151-2 up	up	-40	241 156
Cl 5A84	148-1 PA	P2		Thrombospondin 100%	Thrombospondin	N124 148-1 down 151-1 down 151-2 up	down	-40	233
Cl 2583	151-2 LMA	P5			53BP2 P53-binding protein (53.3%)	148-1 down 151-1 down 151-2 up	down		
Cl 2983 Cl 2881 (same frag; different orientation)	148-1 LMD	P5	335 332		TGF-Beta 2 (53.0%) Kv1.1 n.s.L. (-95%)	N119 148-1 up 151-1 up 151-2 up	up	T7	335 332
Cl 54A#2	148-1 PA	P8		Musculus receptor tyrosin kinase cyclin G	Proto-oncogene tyrosine-protein kinase gene	N126 148-1 down (weak) 151-1 down (weak) 151-2 up (weak)	down	Sp6	220
Cl 63#4	151-2 LMA	P10			Y318 gene (53.8%) 1AC gene (53.8%) Rb susceptibility gene (50%)	N127	up	Sp6	340
Cl 74#2	151-2 LMA	P11#3		86.8% serum & glucocorticoid regulated kinase (sgk)		N120 148-1 up 151-1 down 151-2 up		Sp6	320
Cl 75#1	151-2 LMA	P11#10		87% match sgk			up	Sp6	260
Cl 788#4 match the same gene but diff. frag.	148-1 LMD	P12		92.2% match sgk	protein kinase C-L (57%)			Sp6	270

FIGURE 13B

DD-PCR Primer and PCR size (nt)	mouse homology(%nt)	human homology (%nt)	TGF-beta stimulatory response (12 hr.)	Northern (P-MT)	Cell line
P11-2 c15 (310)	Lysyl oxidase (100%)		↑ ↑ ↑	↑ ↑	N132: 148-1 LMD , 151-1 LM1 down, 151-2 LMB, 151-2 LMC up
P20-23 c19 (850)	Actin binding protein(100%)		↑ ↑	↑ ↑	N142: 148-1 LMD, 151-2 LMA, LMB, MMA up, 151-1 LM1 unchanged
C129-3 (P5) (335)		NMB(79.8%)	↑ ↑	↑ ↑	N119: 148-1 LMD , 151-1 LM1, 151-2 LMA, LMB, LMC, MMA up
P17-3 c18 (1000)	Ubiquitin activating enzyme E1(100%)		↑	↑ ↑	N142: 151-2 LMA down
P20-3 (400)		alpha actinin 3 mRNA (77.5%)	↑ ↑		
P18-12 c13 (1000)	Rat mRNA for P34 protein (89.6%)		↑		
P25-7 c13 (1000)	M.musculus mRNA for P19-protein tyrosine phosphatase (100%)		↑	↑ ↑	148-1LMD up
P19-1 c13 (310)		polymorphic loci in Xq28 (30%)	↑		

FIGURE 13C

DD-PCR primer and PCR size (nt)	mouse (rodent) homology (%nt)	human homology (%nt)	screen 1 P53 stimulatory response (12h. or 24h.)	screen 2 cloned DNA
P1-8 cl10 (1000)		dystrophin gene (50.4%)	P53(+)24 down, down	P53(+)24 down,down
P1-9 cl10 (500)	M. musculus mRNA for cyclin G (86.5%)		P53(+)12 up,up P53(+)24 up,up,up	P53(+)12 up,up,up P53(+)24 up,up,up
P7-4 cl1 (600)	rattus norvegicus spk mRNA (51.3%), rat lung derived L01 C-ras-1 proto-oncogene mRNA (48.4%)	nitric oxide synthase (47.1%)	148-1LMD down P53(+)12 up,up P53(+)24 up,up,up	P53(+)12 up P53(+)24 up
P8-17 cl9 (500)	rat mRNA for cyclin D1 (79.1%)		P53(+)24 up	P53(+)24 up
P9-20 cl3 (850)		HL capons LDLC mRNA (51.8%)	P53(+)12 down P53(+)24 down,down	P53(+)24 down
P11-23 cl2 (800)	syrian hamster gene for cytochrome P-4 (52.5%), rat carbohydrate binding receptor gene (50.6%)		P53(+)24 up,up	P53(+)24 up
P15-8 cl1 (500)	mouse (clone BALB11N) mRNA (47.2%)	PTGS2 gene for prostaglandin endoperoxide synthase-2 (46.6%)	P53(+)24 down	P53(+)24 down,down
P15-14 cl5 (500)			P53(+)12 up P53(+)24 up	P53(+)24 up
P18-23 cl10 (500)			148-1LMD down P53(+)12 down P53(+)24 down	148-1LMD down P53(+)12 down P53(+)24 down

FIGURE 13D

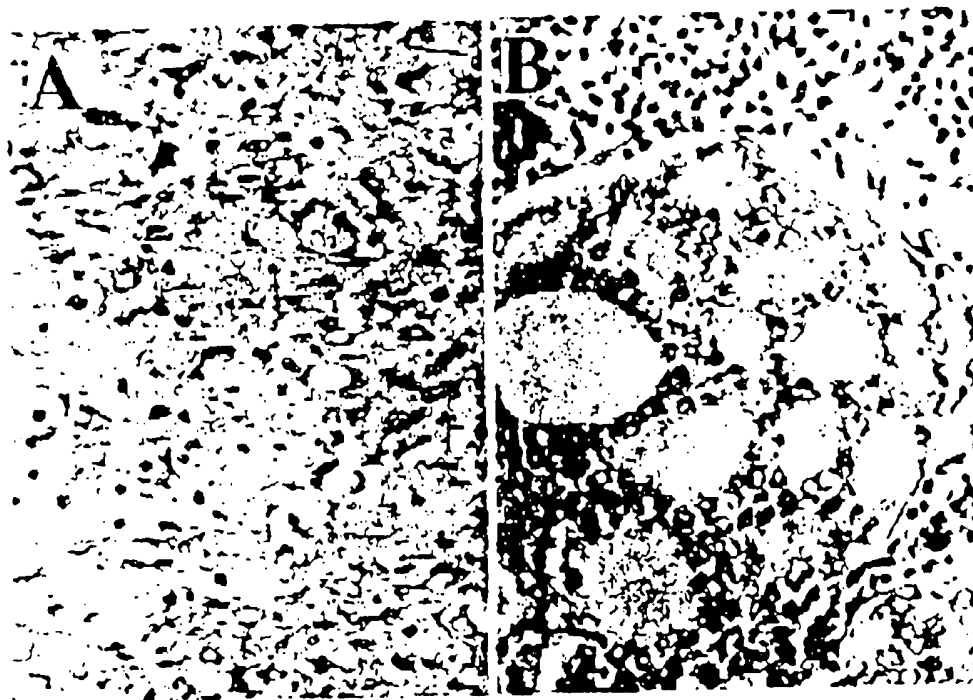


FIGURE 14

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12Q 1/68	A3	(11) International Publication Number: WO 97/18454 (43) International Publication Date: 22 May 1997 (22.05.97)
(21) International Application Number: PCT/US96/18567 (22) International Filing Date: 15 November 1996 (15.11.96) (30) Priority Data: 60/006,838 16 November 1995 (16.11.95) US 08/594,031 30 January 1996 (30.01.96) US (71)(72) Applicant and Inventor: THOMPSON, Timothy [US/US]; 4835 Jason Street, Houston, TX 77096 (US). (74) Agents: REMENICK, James et al.; Baker & Botts, L.L.P., The Warner, 1299 Pennsylvania Avenue, N.W., Washington, DC 20004 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 14 August 1997 (14.08.97)
(54) Title: METHOD FOR IDENTIFYING METASTATIC SEQUENCES (57) Abstract The invention relates to methods for the identification of metastatic sequences. Cells from a cell line or an animal tissue are treated to form a cell line predisposed to metastasis. Treated cells are implanted in an animal of a primary site and incubated for a period of time sufficient for the cells to proliferate and develop metastases at secondary sites. Expressed sequences from cells at the primary and secondary sites are amplified by differential display polymerase chain reaction and compared. Differentially expressed sequences are identical and can be cloned and sequenced. These sequences can be used as probes in the diagnosis of metastatic disorders, as probes to isolate metastatic sequences and as a therapeutic agent.		

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/18567

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

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IPC 6 C12Q

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 94 28129 A (ISIS INNOVATION ;TARIN DAVID (GB)) 8 December 1994 see the whole document ---	1-62
Y	WO 95 19369 A (UNIV VANDERBILT) 20 July 1995 see the whole document ---	1-62
Y	WO 86 03226 A (WHITEHEAD BIOMEDICAL INST) 5 June 1986 see the whole document ---	1-62
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- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

Date of the actual completion of the international search

30 May 1997

Date of mailing of the international search report

23. 06. 97

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Hagenmaier, S

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/18567

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCIENCE, vol. 257, no. 5072, 14 August 1992, pages 967-971, XP000508268 LIANG P ET AL: "DIFFERENTIAL DISPLAY OF EUKARYOTIC MESSENGER RNA BY MEANS OF THE POLYMERASE CHAIN REACTION" see the whole document ---	1-62
A	THE JOURNAL OF HISTOCHEMISTRY AND CYTOCHEMISTRY, vol. 42, no. 4, 1 April 1994, pages 505-511, XP000575423 WOOD D P ET AL: "SENSITIVITY OF IMMUNOHISTOCHEMISTRY AND POLYMERASE CHAIN REACTION IN DETECTING PROSTATE CANCER CELLS IN BONE MARROW" see the whole document ---	1-62
A	CANCER RESEARCH, vol. 52, December 1992, pages 6966-6968, XP002032010 LIANG ET AL.: "Differential display and cloning of messenger RNAs from human breast cancer versus mammary epithelial cells" see the whole document ---	1-62
A	PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, TORONTO, MAR. 18 - 22, 1995, vol. 36, 18 March 1995, AMERICAN ASSOCIATION FOR CANCER RESEARCH, page 266 XP002019344 ROBSON C N ET AL: "IDENTIFICATION OF PROSTATIC ANDROGEN REGULATED GENES USING THE DIFFERENTIAL DISPLAY TECHNIQUE" see the whole document ---	1-62
A	ONKOLOGIE, vol. 18, no. SUPPL. 03, 1 November 1995, pages 2-7, XP000576595 SCHLAG P M ET AL: "FRUEHERKENNUNG VON KREBS MIT HILFE VON MOLEKULARBIOLOGISCHEN MARKERN" see the whole document ---	1-62
A	PNAS, vol. 87, October 1990, pages 7678-7682, XP002031777 WELCH ET AL.: "Transforming growth factor beta stimulates mammary adenocarcinoma cell invasion and metastatic potential" see the whole document ---	1-62

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/18567

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CELL, vol. 56, 1989, pages 917-930, XP002031778 THOMPSON ET AL.: "Multistage carcinogenesis induced by ras and myc oncogenes in a reconstituted organ" see the whole document ---	1-62
A	FORTH,HENSCHLER,RUMMEL: "Allgemeine und spezielle Pharmakologie und Toxikologie" 1987, WISSENSCHAFTSVERLAG,MANNHEIM,DE XP002031779 149990 see page 716 - page 723 ---	1-62
P,X	WO 96 30389 A (MILLENIUM PHARM INC) 3 October 1996 see page 19 see the whole document -----	1-62

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/ 18567

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 31,32,61,62
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims (as far as in vivo methods are concerned) are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 96/18567

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WO 9428129 A	08-12-94	AU 6802294 A EP 0700436 A	20-12-94 13-03-96
WO 9519369 A	20-07-95	AU 1831795 A	01-08-95
WO 8603226 A	05-06-86	AU 5197986 A EP 0203970 A JP 62501399 T	18-06-86 10-12-86 11-06-87
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